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13. ABSTRACT *(Maximum 200 Words)*

Our preliminary data indicated the presence of a breast carcinoma metastasis suppressor genes on human chromosome 11. The goal was to identify and begin characterizing the gene(s) responsible. Two approaches were proposed – (1) introduction of smaller pieces of chromosome 11 into cells with assessment of metastatic potential; and (2) identification of differentially expressed mRNA in metastasis suppressed cells. Progress using Approach #1 was not successful.

Approach #2 was successful. We identified three novel cDNAs using differential display which were more highly expressed in the metastasis-suppressed neo11/435 hybrids. Moreover, one of those candidates, BRMS1, significantly suppressed metastasis in two human breast carcinoma cell lines when transfected and constitutively expressed. BRMS1 maps to 11q13, a site commonly involved in late-stage breast carcinoma. The mechanism of action appears to be novel, but was not determined during the funding period.

In summary, we accomplished the stated ultimate objective for DAMD-17-96-1-6152. Results generated from DAMD-17-96-1-6152 were used to successfully compete for funding from the National Cancer Institute to follow-up on these exciting findings.

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FOREWORD

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(4) TABLE OF CONTENTS

(5) INTRODUCTION

Most cancer deaths are due to metastasis. The goal of this grant was to identify metastasis-controlling genes for human breast cancer. The research plan was based upon our finding that microcell-mediated transfer of chromosome 11 into MDA-MB-435 results in nearly complete suppression of metastasis without suppressing tumorigenicity. Our global objectives were: (1) to identify the gene(s) on chromosome 11 responsible for metastasis suppression; and (2) to assess whether chromosome 11 suppresses metastasis of another independently-derived breast cancer cell line. Four Specific Aims were proposed: Specific Aim #1: Map the gene(s) responsible for suppressing metastasis of MDA-MB-435 to within 5 Mb by using MMCT with radiation-deletion variants of chromosome 11. Specific Aim #2: Stably introduce intact neo-tagged human chromosome 11 into MDA-MB-231 cells by MMCT. Specific Aim #3: Identify metastasis-associated genes in neo11/MDA-MB-435 cells using differential display and/or subtraction hybridization. Specific Aim #4: Determine whether specific genes are metastasis-suppressors in MDA-MB-435 and MDA-MB-231 cells.

(6) BODY

Background – Positional cloning has been used to identify a number of tumor-suppressor genes (e.g., WT1, Rb, FHIT) and genes for mutations that predispose cancer susceptibility (e.g, NF1, APC) (reviewed in [1]). As mapping nears completion, detection of mutations among cancer families confirms a particular gene's role as a tumor suppressor. Since mutations are relatively rare, equally strong evidence for a role in cancer etiology is required. Thus, positional cloning is reasonable if strong, well-characterized pedigrees are available. However, determining roles for genes in sporadic tumors or progression-associated genes (e.g., metastasis-controlling) has been difficult because of tumor heterogeneity, genetic instability and the huge number of experiments necessary to prove causality. This is further complicated for multigenic, complex phenotypes, like metastasis. Simply, the statistical likelihood for identifying a specific gene over the immense background of genetic instability typical of late-stage tumors is difficult. Thus, alternative approaches were required.

MMCT (microcell-mediated chromosome transfer) has provided functional evidence for tumor suppressor genes when other approaches have failed [2;3]. The functional data provided the necessary information for successful mapping of the genes responsible [4-20]. We hypothesized that this approach might also be useful for identifying metastasis suppressor genes.

The strategies we proposed for identifying metastasis-controlling genes in human breast cancer were based upon those we used to identify novel metastasis-suppressor genes in human melanoma [21-25]. At the time the original proposal was submitted, we had obtained preliminary data demonstrating that introduction of an intact chromosome 11 significantly suppressed metastasis of the human breast carcinoma cell line MDA-MB-435. This result was confirmed and published shortly after this grant was received [26].

The ultimate objective of the proposed studies was to identify the gene(s) responsible. Two concurrent approaches were outlined. First, progressively smaller fragments of neo-tagged human chromosome 11 were to be introduced into MDA-MB-435 by MMCT. By evaluating regions of overlap for chromosomal fragments present/absent in suppressed/non-suppressed hybrids, the location of the putative metastasis-suppressor gene(s) would be defined. The second approach was to use differential display [27;28] and/or subtractive hybridization [29;30]. Once candidate genes were identified, transfections and testing for metastasis in appropriate animal models would confirm that a *bona fide* metastasis-suppressor gene had been cloned.

The second major objective of DAMD-17-1-96-6152 was to demonstrate that the introduction of chromosome 11 (or candidate metastasis suppressor genes) into another metastatic human breast

carcinoma also suppresses metastasis.

Results – We were successful in achieving the goals outlined in this grant. Most of the studies have been published; so, the descriptions outlined below will be abbreviated. Only one objective was not achieved. We were not able to introduce chromosome 11 into another human breast carcinoma cell line. The reason was simple – until the last year of the grant, we were not able to obtain another metastatic cell line. We obtained several cells from other laboratories which were reportedly metastatic, but those variants were nonmetastatic when tested in our laboratory. Some were not even tumorigenic! By the time we validated that the MDA-MB-231 cells were metastatic, we had candidate genes in-hand. So, we opted to pursue that line of investigation first. Indeed, we found that the candidate gene suppressed metastasis of both human breast carcinoma cell lines. By inference, then, we would predict that introduction of chromosome 11 would likewise lead to metastasis suppression.

Introduction of chromosome 11 suppresses metastasis of human breast carcinoma MDA-MB-435

The final studies for this initial finding were completed with funds from this grant. USAMRMC funds were used also for publication costs. This work was done in collaboration with Dr. Bernard Weissman at the University of North Carolina – Chapel Hill, in conjunction with his graduate student, Karen Phillips [26].

Role of Kai1 in human breast cancer metastasis

Just prior to the original submission of this grant, Kai1 was discovered as a prostate metastasis suppressor gene in rat tumors [31]. Based upon its mapping to chromosome 11 in humans, we hypothesized that it may be responsible for metastasis suppression in the neo11/435 hybrids. As a first study, we identified *Kai1* as a marker correlating with breast cancer metastasis at the mRNA level [32]. Rose Yang was a graduate student in the laboratory of Dr. Lisa Wei, a co-investigator who decided to move prior to the grant funding.

Subsequently, we transfected Kai1 into MDA-MB-435 cells and demonstrated metastasis suppression, albeit at a modest level [33]. This was the first report that Kai1 could suppress metastasis in human cancer. Subsequently, others have shown that Kai1 could suppress metastasis [34] and that expression levels correlate with tumor progression toward malignancy [35-53].

Introduction of chromosome 11 fragments into MDA-MB-435 cells

One of our planned approaches to map/clone the gene(s) on human chromosome 11 involved introduction of radiation deletion variants [54-57]. We labored with this approach for several months, but abandoned it based upon two technical considerations – (1) the time required to define the deleted regions was beyond the scope of this proposal; and (2) retrofitting [58] large-insert vector forms (P1, PAC, BAC, YAC ...) with selectable markers did not work as well as expected. We also performed some studies in collaboration with Dr. Jane Fountain from the University of Southern California on chromosome 11-encoded melanoma tumor suppressor genes utilizing this approach. Mice have been injected with some of the melanoma cells into which the chromosome 11 fragments were introduced, but we have decided to forego this approach in the melanoma project as well.

Protein kinase C-delta promotes metastasis of breast and mammary tumors

In collaboration with Dr. Susan Jaken, we found (using a series of rat mammary adenocarcinoma cell lines which I had developed [59-61]) that PKCδ activity was proportionate to metastatic potential. To test whether PKCδ was responsible for controlling metastasis, cells were transfected with a constitutive expression vector and measured for tumorigenicity and metastasis

[62]. A subsequent study was done using dominant negative regulatory domains controlled by the tetracycline-inducible expression system [63]. Both studies showed increased metastasis when PKCδ was overexpressed and activated.

The melanoma metastasis suppressor gene, KiSS1, suppresses MDA-MB-435 metastasis

We cloned a melanoma metastasis suppressor, KiSS1, which maps to a region on chromosome 1q [24]. This chromosomal region has also been implicated in breast cancer progression [64]. This led to the hypothesis that KiSS1 expression may suppress metastasis of human breast carcinomas as well. Transfection of KiSS1 into MDA-MB-435 cells resulted in significant suppression of metastasis of these cells [65].

MEK1 induces tumorigenicity and metastasis of NIH3T3 cells

Several prior studies had demonstrated that transfection of constitutively active *ras* into untransformed cells could confer tumorigenic and metastatic phenotypes [66-68]. In collaboration with Dr. Alessandro Alessandrini, we tested that hypothesis that transfection with a constitutively active downstream effector of *ras*, MEK1, could similarly confer tumorigenic and metastatic capacity upon untransformed cells. Transfection of constitutively active MEK1 indeed resulted in transformation of NIH3T3 cells as well as acquisition of metastatic potential [69].

Reviews

Several review articles and position papers have been published during the time frame of this grant [64;70-73]. Results from the studies funded by this grant were instrumental in the conclusions reported therein. Results obtained from the research supported by DAMD-17-96-1-6152 influenced our opinions about suitability of tumor progression markers and upon the steps of metastasis impacted by metastasis suppressor genes.

Cloning of metastasis suppressor genes

The primary objective of DAMD-17-96-1-6152 was to identify and clone a human breast cancer metastasis suppressor gene. Parallel approaches were undertaken with limited success. Prior progress reports detail the trials and tribulations of those approaches. Eventually, differential display was used to clone a novel human gene, designated *BRMS1*, which maps to chromosome 11q13 [74]. Details regarding the discovery and initial characterization of BRMS1 can be found in the recently published paper. A mechanism of action for BRMS1 has not yet been determined, but some preliminary data has been obtained. A survey of multiple metastasis-associated phenotypes using *in vitro* assays was done in order to identify the step(s) in metastasis affected by BRMS1. Except for a modest (30%) decrease in motility using Boyden chamber and wound healing assays [75], the cells were nearly identical. Invasiveness, MMP production,

Table 1: Differentially expressed genes in neo11/435 and MDA 435 cells identified by cDNA microarray

Gene	Difference (fold)
Higher in MDA 435 (Metastatic)	
Osteopontin	14.4
Ras-homolog (D78132)	6.8
Osteonectin	6.1
Calcyclin	6.1
Casein kinase	4.2
Tenascin C	3.8
Gelsolin	3.6
IAP	3.2
NMB	2.2
Profilin	2.1
Higher in neo11/435 (Nonmetastatic)	
α1 antichymotrypsin	4.4
PEDF	4.2
TSC-22	3.5
αB crystallin	3.2
KAI1	2.8
Cathepsin D	2.0

adhesion to extracellular matrix components and *in vitro* growth were unaffected. Interestingly, in collaboration with Dr. Henry Donahue, we found that BRMS1 restores homotypic gap junctional intercellular communication [76]. Studies are underway to extend these initial observations.

Microarrays allow simultaneous analysis of gene expression profiles that accompany various disease states [77-79]. Therefore, we utilized the recently developed microarray technologies (Affymetrix oligonucleotide and Incyte cDNA microarrays) to compare MDA 435 and neo11/435 cells (**Table 1**). These were done in collaboration with Dr. Graham Casey (Cleveland Clinic Foundation). A limited number of differences >2-fold were observed, but most were verified in replicate, independently-performed assays as well as by quantitative real-time PCR and/or Northern blotting [80]. Expression of only one known gene, osteopontin (OPN), differed by more than 10-fold. Several other genes were identified that are believed to be either positive (osteonectin (OSN) and calcyclin) or negative (Kai1) regulators of metastasis. We are preparing a manuscript describing these results and have begun to develop follow-up studies for which funding will be applied.

(7) KEY RESEARCH ACCOMPLISHMENTS

- Discovery of a novel human breast carcinoma metastasis suppressor gene, BRMS1

- Demonstration that MEK1 is an oncogene and metastasis-promoting gene

- Demonstration that PKCδ is a metastasis-promoting gene

- Demonstration that Kai1 can inhibit human breast cancer metastasis in a nude mouse model

- Demonstration that KiSS1 can inhibit human breast cancer metastasis in a nude mouse model

(8) REPORTABLE OUTCOMES

Full-length Papers (Only reprints not submitted with previous progress reports are included in the appendix)

Phillips, K.*, Welch, D.R.*, Miele, M.E., Lee, J.-H., Wei., L.L., and Weissman, B.E. Suppression of MDA-MB-435 breast carcinoma cell metastasis following the introduction of human chromosome 11. *Cancer Research* (1996) 56: 1222-1227. * *Contributed equally to this work.*

Welch, D.R. Technical considerations when studying cancer metastasis *in vivo*. (1997) *Clinical and Experimental Metastasis* 15(3): 272-306.

Lee, J-H. and Welch, D.R. (1997) Suppression of metastasis in human breast carcinoma MDA-MB-435 cells following transfection with the metastasis-suppressor gene, *KISS-1*. *Cancer Research* 57: 2284-2287.

Yang, X., Welch, D.R., Phillips, K.K., Weissman, B.E., Wei, L.L. (1997) KAI-1, a putative marker for metastatic potential in human breast cancer. *Cancer Letters* 119: 149-155.

Phillips, K.K., White, A.E., Hicks, D.J., Welch, D.R., Barrett, J.C., Wei, L.L. and Weissman, B.E. (1998) Suppression of metastasis in the MDA-MB-435 model system correlates with increased expression of KAI-1 protein. *Molecular Carcinogenesis* 21: 111-120.

Welch, D.R. and Goldberg, S.F. (1997) Molecular mechanisms controlling human melanoma progression and metastasis. *Pathobiology* 65: 311-330

Welch, D.R. and Wei, L.L. (1998) Molecular control of breast cancer progression and metastasis.

Endocrine Related Cancers 5: 155-196.

Welch, D.R., Harms, J.F., Goldberg, S.F., Meehan, W.J., Seraj, M.J., Leonard, T.O., Samant, R.S., Miele, M.E., Lee, J.-H. and Hicks, D.J. (1999) Identifying and characterizing metastasis-suppressor genes in human cancer. *Biological Approaches to Cancer Therapy* 1: 32-38.

Meehan, W.J., Spencer, J.P.E., Rannels, D.E., Welch, D.R., Knobbe, E.T., and Ostrander, G.K. (1999) Hydrogen peroxide induces oxidative damage in rat type II pulmonary epithelial cells. *Environmental and Molecular Mutagenesis* 33: 273-278.

Kiley, S.C., Clark, K.J., Duddy, S.K., Welch, D.R. and Jaken, S. (1999) Protein kinase C δ involvement in mammary tumor cell metastasis. *Cancer Research* 59: 3230-3238.

Kiley, S.C., Clark, K.J., Duddy,S.K., Welch, D.R. and Jaken, S. (1999) Increased protein kinase C-δ in mammary tumor cells: relationship to transformation and metastatic progression. *Oncogene* 18: 6748-6757.

Welch, D.R. and Rinker-Schaeffer, C.W. (1999) What defines a useful marker of metastasis in human cancer? [Invited editorial]. *Journal of the National Cancer Institute* 91: 1351-1353.

Welch, D.R., Sakamaki, T., Pioquinto, R., Leonard, T.O., Goldberg, S.F., Hon, Q., Erikson, R.L., Rieber, M., Strasberg-Rieber, M., Hicks, D.J., Bonventre, J.V., Alessandrini, A. (2000) Transfection of constitutively active Mek1 confers tumorigenic and metastatic potentials to NIH3T3 cells. *Cancer Research* 60: 1552-1556.

Welch, D.R., Steeg, P.S., and Rinker-Schaeffer, C.W., (2000) Genetic regulation of human breast carcinoma metastasis. *Biology of Breast Cancer Metastasis* (Current Sciences Ltd.) (In press).

Seraj, M.J.*, Samant, R.S.*, Verderame, M.F., Welch, D.R. Functional evidence for a novel human breast carcinoma metastasis suppressor, *BRMS1*, encoded at chromosome 11q13 * *Contributed equally to this work. Cancer Research* 60: 2764-2769.

Yoshida, B., Welch, D.R. Rinker-Schaeffer, C.W. Metastasis suppressor genes. *Journal of the National Cancer Institute* (In press)

Books and Chapters

Welch, D.R. In vivo cancer metastasis assays. In: *Laboratory Techniques in Biochemistry and Molecular Biology*, Editors: Burger, M.M., Rusciano, D. and Welch, D.R. (In press)

Welch, D.R. Metastasis suppressor genes. In: *Cancer Research: an encyclopedic reference*, Editor: Schwab, M., Springer, Berlin (2000) (In press)

Samant, R.S. and Welch, D.R. BRMS1. In: *Cancer Research: an encyclopedic reference*, Editor: Schwab, M., Springer, Berlin (2000) (In press)

Abstracts

Wei, L.L., Yang, X., Phillips, K., Weissman, B.E., Welch, D.R. Analysis of KAI-1 mRNA expression in human breast cancer cell lines. Proceedings of the American Association for Cancer Research (1996) 37: 529.

Phillips, K. K., Welch, D.R., Miele, M.E., Lee, J.-H., Wei, L.L., and Weissman, B.E. Suppression of MDA-MB-435 breast carcinoma cell metastasis following the introduction of human chromosome 11. Proceedings of the American Association for Cancer Research (1996) 37: 568.

Kiley, S., Welch, D.R. and Jaken, S. W. Protein kinase Cδ: use of a dominant-negative strategy to study its contribution to metastatic progression in mammary epithelial cells. Proceedings of the American Association for Cancer Research (1996) 37: 1059.

Lee, J.-H., Goldberg, S.F., Hicks, D.J., Welch, D.R. Suppression of human breast carcinoma MDA-MB-435 tumor growth and metastasis by KiSS-1. Proceedings of the American Association for Cancer Research (1997) 38: 545.

Kiley, S., Goodnough, M., Clark, K., Welch, D.R., Jaken, S. Protein kinase C δ promotes mammary tumor progression. Proceedings of the American Association for Cancer Research (1997) 38: 2500.

Jaken, S., Kiley, S., Clark, K., Goodenough, M., Welch, D. and Medina, D. Protein kinase C in mammary carcinogenesis. Vermont Cancer Meetings (1997). 1:1.

Jaken, S., Kiley, S.C., Medina, D. Welch, D.R. Protein kinase C in mammary carcinogenesis. An Era of Hope – U.S. Army Medical Research and Materiel Command Breast Cancer Research Program (1997) 2: 411.

Lee, J.-H., Hicks, D.J., Goldberg, S.F. Welch, D.R. Suppression of human breast carcinoma MDA-MB-435 metastasis by the melanoma metastasis-suppressor gene, KiSS-1. An Era of Hope – U.S. Army Medical Research and Materiel Command Breast Cancer Research Program (1997) 2: 715.

Kiley, S., Goodnough, M, Clark, K., Welch, D.R., Jaken, S. Dominant negative protein kinase C-δ inhibits the metastatic progression of mammary tumor cells in vivo. Proceedings of the American Association for Cancer Research (1998) 39: 534

Alessandrini, A. and Welch, D.R., Constitutively active MEK1 induces metastatic potential in NIH-3T3 cells. Cold Spring Harbor Meeting – Cancer Genetics and Tumor Suppressor Genes. (1998) p.23.

Alessandrini, A. and Welch, D.R., Constitutively active MEK1 induces metastatic potential in NIH-3T3 cells. Journal of Biomolecular Structure and Dynamics (1998) 16: 149.

Alessandrini, A. and Welch, D.R. Transfection with constitutively active Mek1 confers tumorigenic and metastatic potential to NIH-3T3 cells. VII International Congress of the Metastasis Research Society (1998)

Kiley, S.C., Clark, K., Welch, D.R., and Jaken, S. Dominant-negative protein kinase C-δ inhibits cytoskeletal-dependent processes in metastatic cells. American Society of Biochemistry and Molecular Biology – Protein kinase C and cellular function (October 9-12, 1998; Lake Tahoe, CA).

Alessandrini, A. and Welch, D.R. Transfection with constitutively active Mek1 confers tumorigenic and metastatic potential to NIH-3T3 cells. Proceedings of the American Association for Cancer Research (1999) 40: 1312.

Rieber, M., Welch, D.R., and Rieber, M.S. Differential gene expression associated with suppression of metastatic melanoma cell spreading and proliferation on adhesion-restrictive substrates. Proceedings of the American Association for Cancer Research (1999) 40: 491.

Seraj, Md. J., Samant, R.S., Verderame, M.F., Hicks, D.J., Sakamaki, T., Hwang, C.K. Weissman, B.E., and Welch, D.R. Identification of breast cancer metastasis-suppressor genes from metastasis-suppressed chromosome 11/MDA-MB-435 hybrids. Proceedings of the

American Association for Cancer Research (1999) 40: 689.

Welch, D.R. Genetic regulation of cancer metastasis. 8th Annual Meeting of the Japanese Association for Metastasis Research (1999) 8: 36.

Welch, D.R., Harms, J.F., Goldberg, S.F., Meehan, W.J., Seraj, M.J., Leonard, T.O., Samant, R.S., Miele, M.E., Lee, J.H., Hicks, D.J. Identifying and characterizing metastasis-suppressor genes in human cancer. Proceedings of the First BACT International Symposium (1999) 1: 32-38.

Welch, D.R., Seraj, M.J., Samant, R.S., Leonard, T.O., Harms, J.F., Verderame, M.F. BrMS1 – A human breast cancer metastasis-suppressor gene encoded on chromosome 11q13.1-q13.2. *Clinical Cancer Research* 5:66.

Samant, R.S., Seraj, M.J., Meehan, W.J., Harms, J.F., Leonard, T.O., Shevde, L.A., Sakamaki, T., Winter, C.R., Verderame, M.F., Welch, D.R. BrMS1 — a human breast carcinoma metastasis-suppressor gene. Proceedings of the American Association for Cancer Research (2000) 41: 1053

Samant, R.S., Debies, M.T., Seraj, M.J., Verderame, M.F., Welch, D.R. Genomic organization and chromosomal localization of the breast metastasis suppressor gene [BRMS1]. Proceedings of the American Association for Cancer Research (2000) 41: 1461.

Saunders, M.M., Seraj, M.J., Yellowley, C.E., Hoke, A., Welch, D.R., and Donahue, H.J. Gap junctional intercellular communication is restored in metastasis-suppressed breast carcinoma cells. Proceedings of the Federation of the Societies of Experimental Biology 2000 (in press).

Samant, R.S., Seraj, M.J., Shevde, L.A., Sakamaki, T., Meehan, W.J., Leonard, T.O., Harms, J.F., Winter, C.R.., Debies, M.T., Verderame, M.F., and Welch, D.R. BRMS1 – a novel human breast cancer metastasis-suppressor gene. 3rd annual regional cancer center consortium for biological therapy of cancer.3: 15.

Presentations

2000   *BRMS1 - a human breast cancer metastasis-suppressor gene encoded on chromosome 11q13.1-q13.2.* Era of Hope DOD Breast Cancer Research Program's Meeting, Atlanta, GA (6/9)

Chromosome 6 blocks growth of human melanoma metastases at the secondary site. AACR Special Conference on Melanoma: Basic Biology and Immunological Approaches, The Woodlands, TX (5/6)

Chromosome 6-melanoma hybrids spread to lung but do not proliferate, Innovations in Biological Therapy of cancer "2000" 3rd Annual regional cancer center consortium for biological therapy of cancer. Hershey, PA (2/25).

Strategies to elucidate the genetics of neoplastic progression and metastasis, Molecular Aspects of Growth Control and Apoptosis, Caracas Venezuela (1/27)

In vivo and in vitro methods to study cancer metastasis, Molecular Aspects of Growth Control and Apoptosis, Caracas Venezuela (1/28)

What defines a clinically useful marker of cancer metastasis? Molecular Aspects of Growth Control and Apoptosis, Caracas Venezuela (1/29)

1999   *Metastasis overview and metastasis genes,* Fall Inland Northwest Cancer Conference –

Research & Clinical Practice: Bridging the Gap (11/6)

Putative Metastasis-suppressor genes in human melanoma, AACR Special Conference – Molecular Aspects of Metastasis, Snowmass, CO (9/23)

Genes controlling metastasis in human melanoma and breast carcinoma, Japanese Association for Metastasis Research, Tokyo Japan (5/24)

Genetics of cancer metastasis: prospects for therapeutic intervention , 1ˢᵗ International BACT Symposium/ 10ᵗʰ Annual Symposium on the Biological Approaches to Cancer Treatment, Nagoya Japan (5/22)

Identification of breast cancer metastasis-suppressor genes from metastasis-suppressed chromosome 11/MDA-MB-435 hybrids, Integrative Biosciences Symposium, State College, PA (5/7)

Host-tumor interactions in cancer invasion and metastasis: Genetic Implications. American Association for Cancer Research Annual Meeting (4/14)

Transfection with constitutively active Mek1 confers tumorigenic and metastatic potential to NIH-3T3 cells, American Association for Cancer Research Annual Meeting (4/12)

1998   *Genetic regulation of melanoma and breast tumor metastasis,* VII International Congress of the Metastasis Research Society, San Diego, CA (10/8)

1997   *Suppression of human breast carcinoma MDA-MB-435 tumor growth and metastasis by KiSS-1*, An Era of Hope (U.S. Army Medical Research and Materiel Command – Breast Cancer Program), Washington, D.C. (11/2)

Identification of metastasis-suppressor genes in human cancer 50ᵗʰ Annual Symposium on Fundamental Cancer Research, M.D. Anderson Cancer Center, (10/31)

Isolation and initial characterization of KiSS-1, a human metastasis-suppressor gene, Cold Spring Harbor/Frederick Cancer Research Center Symposium on Cancer Genetics and Tumor suppressor genes (6/13)

Invited Lectures

2000   *Metastasis suppressor genes in human cancer: from discovery to mechanism of action.* University of New Mexico College of Medicine (6/3)

Metastasis Suppressor Genes in Human Cancer, Life Sciences Consortium Graduate Program in Nutrition, Penn State University, February 15.

Molecular mechanisms of cancer metastasis, University of Rochester Cancer Center, January 24

Genetics of breast cancer metastasis, Penn State Cancer Center, January 17

1999   *Molecular regulation of metastasis – opportunities for clinical intervention*, Genzyme Inc., December 6

New targets for treating cancer – metastasis and genomic instability, Council for the Advancement of Science Writing Inc., November 8

Genes that control cancer metastasis, Pennsylvania Cancer Registry, September 29

Identifying and characterizing human metastasis-suppressor genes. Novartis Pharma, May

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Regulation of Metastasis in Human Cancers, University of Tokyo, May 26

Metastasis suppression in human cancer, University of Chicago Cancer Center, April 28

Update on the genetics of cancer metastasis. Hematology/Oncology Grand Rounds, Penn State Geisinger Health System, March 17

Tumor angiogenesis and metastasis: Therapeutic options. Pennsylvania Society of Oncology Nurses, Hershey, PA, February 24

1998   *Cancer Metastasis – Opportunities for Bioengineering Research*, Penn State University, December 8

Molecular mechanisms controlling cancer metastasis. University of Texas-Houston Medical School, November 18

Cancer Research Update, WLBR Radio-Don Bowman Show, August 17

Molecular regulation of melanoma and breast carcinoma metastasis, Wake Forest University Cancer Center, July 28

Molecular mechanisms controlling melanoma and breast carcinoma metastasis. University of Chicago Cancer Center, May 15

Molecular basis of cancer metastasis, Massachusetts General Hospital, March 18

1997   *Identification and characterization of human metastasis-controlling genes*, American Health Foundation, December 5

The molecular basis of cancer metastasis - Spelman College, November 25

KiSS-1, a human metastasis-suppressor gene, University of Texas System Cancer Center - Science Park Research Division, October 30

Breast cancer research – recent findings, Millersville University, October 14

WLBR Radio-Don Bowman Show, *Conquering Cancer with a Quarter-Research Update*, August 19

Identification and possible mechanisms of action of KiSS-1, a novel human metastasis-suppressor gene, Pathology Grand Rounds, University of Rochester, May 30

KiSS-1, a novel human metastasis-suppressor gene, Surgical Grand Rounds, St. Luke's Hospital, Bethlehem, PA, May 28

Involvement of protein kinase C isoforms in human cancer metastasis control, Endocrinology Research Conference, Penn State University College of Medicine, May 22

1996   *Identification and initial characterization of a novel human melanoma metastasis-suppressor gene, KiSS-1*, Roswell Park Cancer Institute, December 11

Identification and initial characterization of KiSS-1, a novel human melanoma metastasis-suppressor gene M.D. Anderson Cancer Center, November 19

Metastasis-suppressor genes: an opportunity for therapeutic intervention?, American Cancer

Society-Commonwealth Division, October 17

Toward a molecular understanding of melanoma metastasis, Penn State University Bioengineering Program, November 13

Virology In-service, Penn State College of Medicine, *What is a tumor cell?*, August 22

WLBR Radio-Don Bowman Show, *Conquering Cancer with a Quarter-Research Update*, August 20

Penn State University Cancer Center, *Are antisense oligonucleotides potentially useful for melanoma therapy?*, June 17

Patent Application

Paperwork to initiate U.S. and Worldwide patent applications were filed for BRMS1 by Penn State University.

Degrees Obtained That Are Supported by this Award –

None

Development of Cell Lines, Tissue or Serum Repositories

Transfectant cell lines described in the papers and abstracts have been made available to anyone requesting them. We have provided them free-of-charge to over 20 laboratories worldwide. In previous progress reports, we also described our efforts to assist Dr. Stephen Ethier in the characterization of SUM breast carcinoma cells.

Informatics

None

Funding Applied for Based upon this Work

RO1-CA87728 was funded and began July 1st 2000.

Employment/research/training Opportunities

Cheol Kyu (Thomas) Hwang, Ph.D.   Postdoctoral Fellow

    Current position: Postdoctoral Fellow NIH

Md. Jabed Seraj, Ph.D.   Postdoctoral Fellow

    Current position: Research Associate, University of Virginia

Toshiyuki Sakamaki, Ph.D.   Postdoctoral Fellow

Rajeev S. Samant, Ph.D.   Postdoctoral Fellow

(9) CONCLUSIONS

Our preliminary data indicated the presence of one or more breast carcinoma metastasis suppressor genes on human chromosome 11. The goal of this program was to identify and begin characterizing the gene(s) responsible. Two parallel approaches were proposed – (1) introduction of smaller pieces of chromosome 11 into cells with assessment of metastatic potential; and (2) identification of differentially expressed mRNA in metastasis suppressed cells. Progress using Approach #1 was frustrating and not productive. While we attempted several variations on the approach, none were successful.

Approach #2 was successful. We identified three novel cDNAs using differential display which are more highly expressed in the metastasis suppressed neo11/435 hybrids. Moreover, one of those candidates, BRMS1, significantly suppresses metastasis in two human breast carcinoma cell lines when transfected and constitutively expressed. BRMS1 maps to 11q13, a site commonly involved in late-stage breast carcinoma. The mechanism of action appears to be novel, but has not been determined during the funding period.

In short, we accomplished the stated ultimate objective for DAMD-17-96-1-6152. Success required us to modify the experimental strategy, but the general tenor of the experimental outline was retained. Results generated from DAMD-17-96-1-6152 were used to successfully compete for funding from the National Cancer Institute to follow-up on these exciting findings.

(10) REFERENCES

1. E. J. Stanbridge, *Annu.Rev.Genet.* 24, 615-657 (1990).

2. M. J. Anderson and E. J. Stanbridge, *FASEB J.* 7, 826-833 (1993).

3. J. D. Hunt, *Anal.Biochem.* 238, 107-116 (1996).

4. P. Chen, N. Ellmore, B. E. Weissman, *Molec.Cell.Biol.* 14, 534-542 (1994).

5. P. J. Saxon, E. S. Srivatsan, E. J. Stanbridge, *EMBO J.* 5, 3461-3466 (1986).

6. W. E. Loh et al., *Proc.Natl.Acad.Sci.(USA)* 89, 1755-1759 (1992).

7. J. M. Trent et al., *Science (Wash.D.C.)* 247, 568-571 (1990).

8. S. L. Church et al., *Proc.Natl.Acad.Sci.(USA)* 90, 3113-3117 (1993).

9. D. Gioeli, K. Conway, B. E. Weissman, *Cancer Res.* 57, 1157-1165 (1997).

10. M. Koi, P. W. Lamb, L. Filatov, A. P. Feinberg, J. C. Barrett, *Cytogenet.Cell Genet.* 76, 72-76 (1997).

11. H. Kuramochi et al., *Prostate* 31, 14-20 (1997).

12. M. Reiss, V. Santoro, R. R. De Jonge, V. F. Vellucci, *Cell Growth Differ.* 8, 407-415 (1997).

13. N. L. England et al., *Carcinogenesis* 17, 1567-1575 (1996).

14. W. B. Coleman et al., *Molec.Carcinog.* 13, 220-232 (1995).

15. C. M. Ewing et al., *Cancer Res.* 55, 4813-4817 (1995).

16. H. Ohmura et al., *Jpn.J.Cancer Res.* 86, 899-904 (1995).

17. M. Theile et al., *Oncogene* 10, 439-447 (1995).

18. H. Kon et al., *Oncogene* 16, 257-263 (1998).

19. G. P. Robertson et al., *Proc.Natl.Acad.Sci.USA* 95, 9418-9423 (1998).

20. Casey, G., Plummer, S. J., Simmons, J. A., Adams, L., and Paris, M. J. Identification of a novel breast cancer growth suppressor gene on chromosome 17. U.S.Army Medical Research and Materiel Command Era of Hope Meeting 1, 525-526. 1997.

21. D. R. Welch et al., *Oncogene* 9, 255-262 (1994).

22. H. Jiang et al., *Oncogene* 10, 1855-1864 (1995).

23. J.-H. Lee and D. R. Welch, *Intl.J.Cancer* 71, 1035-1044 (1997).

24.   J.-H. Lee et al., *J.Natl.Cancer Inst.* 88, 1731-1737 (1996).

25.   M. E. Miele et al., *Intl.J.Cancer* (in press), (1997).

26.   K. K. Phillips et al., *Cancer Res.* 56, 1222-1226 (1996).

27.   P. Liang, L. Averboukh, A. B. Pardee, *Nucleic Acids Res.* 21, 3269-3275 (1993).

28.   P. Liang and A. B. Pardee, *Science (Wash.D.C.)* 257, 967-971 (1992).

29.   F. M. Ausubel et al., Current protocols in molecular biology (John Wiley & Sons, New York, 1990).

30.   J. T. Hutchins, R. J. Deans, M. S. Mitchell, C. Uchiyama, J. Kan-Mitchell, *Cancer Res.* 51, 1418-1425 (1991).

31.   J.-T. Dong et al., *Science (Wash.D.C.)* 268, 884-886 (1995).

32.   X. H. Yang, D. R. Welch, K. K. Phillips, B. E. Weissman, L. L. Wei, *Cancer Lett.* 119, 149-155 (1997).

33.   K. K. Phillips et al., *Molec.Carcinog.* 21, 111-120 (1998).

34.   A. Takaoka et al., *Jpn.J.Cancer Res.* 89, 397-404 (1998).

35.   M. Adachi et al., *Cancer Res.* 56, 1751-1755 (1996).

36.   J. T. Dong et al., *Cancer Res.* 56, 4387-4390 (1996).

37.   X. Z. Guo et al., *Cancer Res.* 56, 4876-4880 (1996).

38.   X. Guo et al., *Cancer Res.* 56, 4876-4880 (1996).

39.   T. Ueda et al., *American.Journal of Pathology.* 149, 1435-1440 (1996).

40.   X. Z. Guo et al., *Cancer Res.* 58, 753-758 (1998).

41.   A. Takaoka et al., *Oncogene* 16, 1443-1453 (1998).

42.   M. Higashiyama et al., *Cancer* 83, 466-474 (1998).

43.   Y. Hinoda et al., *Cancer Lett.* 129, 229-234 (1998).

44.   H. Friess et al., *Int.J.Cancer* 79, 349-355 (1998).

45.   M. Adachi et al., *J.Clin.Oncol.* 16, 1397-1406 (1998).

46.   J. Geradts et al., *Am.J.Pathol.* 154, 1665-1671 (1999).

47.   H. C. Sun, Z. Y. Tang, G. Zhou, X. M. Li, *J.Exp.Clin.Cancer Res.* 17, 307-311 (1998).

48.   J. M. Ward et al., *Pathol.Int.* 48, 87-92 (1998).

49.   Y. Yu et al., *Clin.Cancer Res.* 3, 1045-1049 (1997).

50.   K. Tagawa et al., *Japanese Journal of Cancer Research (Amsterdam)* 90, 970-976 (1999).

51.   D. P. Lombardi et al., *Cancer Res.* 59, 5724-5731 (1999).

52.   A. Burchert et al., *Br.J.Haematol.* 107, 494-504 (1999).

53.   Leonard, T. O., Hicks, D. J., Fountain, J. W., and Welch, D. R. Kai1 suppresses metastasis and tumor growth in the human metastatic melanoma cell line MelJuSo. Proceedings of the American Association for Cancer Research 41, 58. 2000.

54.   S. A. Bader, C. Fasching, G. M. Brodeur, E. J. Stanbridge, *Cell Growth Differ.* 2, 246-255 (1991).

55.   S. F. Dowdy, D. J. Scanlon, C. L. Fasching, G. Casey, E. J. Stanbridge, *Genes, Chromosomes, Cancer* 2, 318-327 (1990).

56.   G. Robertson, A. Coleman, T. G. Lugo, *Cancer Res.* 56, 4487-4492 (1996).

57.   G. P. Robertson, A. Hufford, T. G. Lugo, *Cytogenet.Cell Genet.* 79, 53-59 (1997).

58.   J. E. Mejia and A. P. Monaco, *Genome Res.* 7, 179-186 (1997).

59.   A. Neri, D. R. Welch, T. Kawaguchi, G. L. Nicolson, *J.Natl.Cancer Inst.* 68, 507-517 (1982).

60.   D. R. Welch and G. L. Nicolson, *Clin.Exptl.Metastasis* 1, 317-325 (1983).

61.   D. R. Welch, A. Neri, G. L. Nicolson, *Invasion Metastasis* 3, 65-80 (1983).

62.   S. C. Kiley, K. J. Clark, S. K. Duddy, D. R. Welch, S. Jaken, *Oncogene* 18, 6748-6757 (1999).

63.   S. C. Kiley, K. J. Clark, M. Goodnough, D. R. Welch, S. Jaken, *Cancer Res.* 59, 3230-3238 (1999).

64.   D. R. Welch and L. L. Wei, *Endocrine-related Cancer* 5, 155-197 (1998).

65.   J.-H. Lee and D. R. Welch, *Cancer Res.* 57, 2384-2387 (1997).

66.   R. J. Muschel, J. E. Williams, D. R. Lowy, L. A. Liotta, *American.Journal of Pathology.* 121, 1-8 (1985).

67.   U. P. Thorgeirsson et al., *Molecular & Cellular Biology* 5, 259-262 (1985).

68.   A. F. Chambers and A. B. Tuck, *Crit Rev.Oncog.* 4, 95-114 (1993).

69.   D. R. Welch et al., *Cancer Res.* 60, 1552-1556 (2000).

70.   D. R. Welch and S. F. Goldberg, *Pathobiol.* 65, 311-330 (1997).

71.   D. R. Welch, *Clin.Exptl.Metastasis* 15, 272-306 (1997).

72.   D. R. Welch and C. W. Rinker-Schaeffer, *J.Natl.Cancer Inst.* 91, 1351-1353 (1999).

73.   D. R. Welch, P. S. Steeg, C. W. Rinker-Schaeffer, *Biology of Breast Cancer Metastasis* (In press), (2000).

74.   M. J. Seraj, R. S. Samant, M. F. Verderame, D. R. Welch, *Cancer Res.* 60, 2764-2769 (2000).

75.   S. E. Walther and D. T. Denhardt, *Cell Growth Differ.* 7, 1579-1588 (1996).

76.   Saunders, M. M., Li, Z., Winter, C. R., Welch, D. R., and Donahue, H. J. Metastatic breast carcinoma correlates with a breakdown in gap junction function and expression. Clinical and Experimental Metastasis 8((in press)). 2000.

77.   D. Gerhold, T. Rushmore, C. T. Caskey, *Trends Biochem.Sci.* 24, 168-173 (1999).

78.   J. Khan et al., *Electrophoresis* 20, 223-229 (1999).

79.   R. J. Lipshutz, S. P. Fodor, T. R. Gingeras, D. J. Lockhart, *Nat.Genet.* 21, 20-24 (1999).

80.   Signorelli, K., Welch, D. R., and Casey, G. Gene expression differences between metastatic and non-metastatic breast cancer cell lines using microarray technology. Proceedings of the American Association for Cancer Research . 1999.

(11) APPENDICES

Curriculum Vitae

Journal Articles:

Welch, D.R. and Rinker-Schaeffer, C.W. (1999) *J. Natl. Cancer Inst.* 91: 1351-1353.

Welch, D.R., et al. (2000) *Cancer Research* 60: 1552-1556.

Welch, D.R., et al. (2000) *Biology of Breast Cancer Metastasis* (Current Sciences Ltd.) (In press).

Seraj, M.J.*, Samant, R.S.*, et al. (2000) *Cancer Research* 60: 2764-2769.

Yoshida, B., Welch, D.R. Rinker-Schaeffer, C.W. (2000) *J. Natl. Cancer Inst.* (In press)

Chapters

Welch, D.R. Metastasis suppressor genes. In: *Cancer Research: an encyclopedic reference,* Editor: Schwab, M., Springer, Berlin (2000) (In press)

Samant, R.S. and Welch, D.R. BRMS1. In: *Cancer Research: an encyclopedic reference,* Editor: Schwab, M., Springer, Berlin (2000) (In press)

Abstracts

Welch, D.R., Seraj, M.J., Samant, R.S., Leonard, T.O., Harms, J.F., Verderame, M.F. BrMS1 – A human breast cancer metastasis-suppressor gene encoded on chromosome 11q13.1-q13.2. *Clinical Cancer Research* 5:66.

Samant, R.S., Seraj, M.J., Meehan, W.J., Harms, J.F., Leonard, T.O., Shevde, L.A., Sakamaki, T., Winter, C.R., Verderame, M.F., Welch, D.R. BrMS1 — a human breast carcinoma metastasis-suppressor gene. Proc. Am. Assoc. Cancer Res. (2000) 41: 1053

Samant, R.S., Debies, M.T., Seraj, M.J., Verderame, M.F., Welch, D.R. Genomic organization and chromosomal localization of the breast metastasis suppressor gene [BRMS1]. Proc. Am. Assoc. Cancer Res. (2000) 41: 1461.

Saunders, M.M., Seraj, M.J., Yellowley, C.E., Hoke, A., Welch, D.R., and Donahue, H.J. Gap junctional intercellular communication is restored in metastasis-suppressed breast carcinoma cells. Proc. Federation of the Societies of Experimental Biology 2000 (in press).

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EIN (consulting):   25-1843278 (Welch Consulting)

EDUCATION

1980 - 1984	The University of Texas - Houston, Graduate School of Biomedical Sciences
	Degree: **Ph.D.** (Biomedical Sciences - Tumor Biology)
1976 - 1980	The University of California - Irvine, School of Biological Sciences
	Degree: **B.S.** (Biological Sciences - Cell Biology)

PROFESSIONAL EXPERIENCE

The Pennsylvania State University College of Medicine

7/1997 - present   Associate Professor of Pathology, Jake Gittlen Cancer Research Institute (Tenured 7/99)
Member - Penn State Cancer Center (3/00 - present)
Member - Graduate Faculty
Cell and Molecular Biology Graduate Program (11/91-present)
Graduate Program in Cellular and Molecular Transport Dynamics (12/95- present)
Integrative Biosciences Graduate Program in Molecular Medicine (04/97 - present)
Medical Scientist Training Program (MD/Ph.D.) (04/97 - present)
Integrative Biosciences Graduate Program in Immunobiology (5/98 - present)

12/1990 - 6/1997   Assistant Professor of Pathology, Department of Pathology

Welch Consulting Incorporated

1/2000 - present   President

Glaxo Research Laboratories, Glaxo, Inc.

10/1989 - 5/1990   Research Investigator - Department of Chemotherapy
10/1988 - 10/1989   Senior Scientist III - Department of Chemotherapy

The Upjohn Company

7/1988 - 10/1988   Scientist II - Department of Cancer and Infectious Diseases Research
8/1984 - 6/1988   Scientist I - Department of Cancer and Infectious Diseases Research

The University of Texas - M.D. Anderson Cancer Center

6/1984 - 8/1984   Postdoctoral Fellow in Tumor Biology
5/1982 - 6/1984   Predoctoral Fellow in Tumor Biology, Advisor: Professor Garth L. Nicolson
7/1980 - 5/1982   Research Assistant I - Department of Tumor Biology

The University of California - Irvine

12/1978 - 6/1980   Laboratory Helper - Department of Developmental and Cell Biology
6/1979 - 6/1980   Undergraduate Research (Bio-199), Supervisor: G.L. Nicolson

GRANT SUPPORT (Current)

1993-2001	PHS, NIH **RO1-CA62168**, Metastasis suppressor genes in human cutaneous melanoma., Principal Investigator, 35% effort.
1996-2000	National Foundation for Cancer Research, Mechanisms of melanoma and breast cancer progression, D.R. Welch, Principal Investigator

1998-2001	Genzyme Corporation, Experimental protocol for investigation of anti-invasive/anti-metastatic compounds in human tumor xenografts, D.R. Welch, Principal Investigator.
2000-2003	Neoplastic consequences of a mutator phenotype in human breast epithelial cells: a prospective analysis, Co-investigator (K.A. Eckert), 5% effort
2000-2003	PHS, NIH **RO1-CA87728**, Molecular regulation of breast cancer metastasis, Principal Investigator, 35% effort

GRANT SUPPORT (Completed)

1999-2000	Innovative Biotechnology Research Fund, Model development to foster understanding of the breast cancer-bone connection, Co-Principal Investigator (C. Gay, A. Mastro, D. Welch), 10% effort.
1996-2000	U.S. Army Medical Research Defense Command, **DAMD-17-96-1-6152**, Molecular mechanisms of metastasis suppression in human breast cancer, Principal Investigator, 25% effort.
1997-1999	PHS, NIH **RO1-CA66021**, Dissecting the roles of chromosome 11q genes in human melanoma, J.W. Fountain, Principal Investigator, D.R. Welch, Co-investigator.
1997-1998	Penn State Cancer Center Melanoma Grant (Foreman Foundation), D.R. Welch, Principal Investigator
1997-1998	Penn State Cancer Center Mentored Melanoma Research Grant, D.R. Welch, Mentor; Steven F. Goldberg, P.I.
1994-1995	ISIS Pharmaceuticals, Antisense oligonucleotides and melanoma metastasis, D.R. Welch, Principal Investigator
1995-1996	National Foundation for Cancer Research, Hormonal regulation of breast cancer progression, D.R. Welch, Principal Investigator
1993-1995	The W.W. Smith Charitable Trust, **C-9302**, Metastasis suppressor genes in human malignant melanoma, D.R. Welch, Principal Investigator, 20% effort.
1993-96	PHS, NIH **2PO1-CA40011**, Mitotic modifiers or hormone-dependent breast cancer, D.R. Welch-Principal Investigator-Project 4 "Immune regulation of breast cancer metastasis", A. Manni, Program Director, 15% effort.
1993-1996	PHS, NIH **2PO1-CA40011**, Mitotic modifiers or hormone-dependent breast cancer, A. Manni, Program Director, D.R. Welch, Principal Investigator-Core E "Animal Core", 15% effort.
1993-1994	Four Diamonds Research Award, Invasiveness of human neuroblastoma cells, A. Freiberg, Principal Investigator, D.R. Welch, Co-investigator, 10% effort.
1993-1994	Clinical Investigation Center Research Grant, Do neutrophils from breast cancer patients enhance metastasis?, D.R. Welch, Principal Investigator
1991-1992	The W. W. Smith Charitable Trust, **C-9102**, Chromosomal Suppressors of Malignant Melanoma Progression, D.R. Welch, Principal Investigator, 20% effort.
1986-1989	PHS, NIH **RO1-CA42475**, Biological Modulation of Human Melanoma Cell Invasion, M.J.C. Hendrix, Principal Investigator, D.R. Welch, Co-investigator.

AWARDS AND HONORS

1999	Desert High School, Edwards, A.F.B., California "Wall of Fame" (Outstanding Alumnus Award)
1998	Nominee, "Excellence in Teaching" Award from Medical Student Class of 2000
1994	Finalist, John Hinkle Society Outstanding Research Award
1993	Nominee, John Hinkle Society Outstanding Investigator Award
1987	Outstanding Young Men of America
1984	University Cancer Foundation Annual Giving - Welch Cancer Fellow Fund
1983	John A. Beck Scholarship
1983	The University of Texas Graduate School of Biomedical Sciences Student Research Symposium -- Outstanding Research Award in Pharmacology
1983	Sigma Xi Honorable Mention -- Presentation at the University of Texas Graduate School of Biomedical Sciences Student Research Symposium
1983	Sigma Xi Scientific Research Society
1982-84	PHS, NCI Training Grant, **5-T32 CA-09299**, Training Grant in Molecular Genetics of Cancer, M.D. Anderson, Program Director
1981	Student Representative for the University of Texas System Cancer Center to the University of Texas Board of Regents - August 1981

1981   University Cancer Foundation Board of Visitors: Special Funding for Immunogenicity Collaboration
              Travel Funds

TEACHING

The Pennsylvania State University College of Medicine

1992 - present ● Course Coordinator and Lecturer - The Biology of Neoplasia (Pathology 520), graduate students and
                  residents
1994 - present ● Lecturer - Molecular Biology of Cellular Growth Control (Biology 561), graduate students
1996 - 1998   ● Special Topics in Pathology - Grant Writing Exercise in conjunction with Biology of Neoplasia
1992 - present ● Lecturer - Pharmacology, medical and graduate students
1991 - present ● Lecturer - Principles of Pathology, medical and graduate students
1994 - 1996   ● Lecturer - Molecular Techniques in Comparative Medicine, (CM-508) graduate students
1992 - 1994   ● Lecturer -Molecular Techniques in Pathology, residents
1996   ● Lecturer, Continuing Medical Education Virology In-service

**Osler Institute, Kalamazoo College, University of Texas, University of California, Visiting
Professorships**

1998 - present ● Visiting Professor, University of Chicago Cancer Center, Biology of Neoplasia
1998   ● Visiting Professor - University of Texas-Houston, M.D./Ph.D. Graduate Program
1992 - 1995   ● Lecturer - Neoplasia, Osler Institute Pathology Board Review Course
1986- 1988   ● Lecturer - Senior Seminars, Kalamazoo College
1981- 1983   ● Graduate Teaching Assistant - Immunology I, The University of Texas Graduate School of
                  Biomedical Sciences
1980   ● Head Coordinator and Lecturer - Biology of Heart Disease (Bio 50), University of California at
                  Irvine
1980   ● Student Coordinator - Biology of Heart Disease (Bio 50), University of California at Irvine

TRAINEES

Undergraduate:
    Michelle Bahner, B.S. (Spelman College), Craig T. Basson, M.D., Ph.D. (University of Texas), Carol Czop, M.D.
    (Kalamazoo College), Erin Gestl, B.S. (Penn State), Casey Glass, B.S. (Messiah College), Sandra Hartman, B.S.
    (Messiah College), Karen Hathaway, M.S. (Kalamazoo College), Richard Howrey, M.D. (Kalamazoo College),
    Jeffery W. Mantia, M.D. (Kalamazoo College), Daniel J. Schissel, M.D. (Kalamazoo College), Kevin Solomon,
    B.S. (Messiah College), Jason Spangler, M.D. (University of Pennsylvania), Peter J. Wack, M.D. (Kalamazoo
    College), James Walker, M.D. (Kalamazoo College)

Graduate (Principal advisor – in bold):
    Lynn Brown, B.S., Molecular Physiology Graduate Program, lung injury (Penn State — 1997-present)*
    Antoine Carlisle, D.V.M., Comparative Medicine Graduate Program, Anti-metastasis drug evaluation (Penn State
        — 1998-2000)
    David Drubin, B.S., Molecular Medicine Graduate Program, Nuclear proteinase/ribozymes (1998-present)
    Vincent Gresham, D.V.M.,M.S., Comparative Medicine Graduate Program, *Yersinia* PCR test (Penn State —
        1993-1994)
        Current position: Director of Laboratory Animal Medicine Services, Brooke Army Medical Center
    Debies, Michael T., B.S., Cell & Molecular Biology Program, BRMS1 knockout mouse (Penn State 1999 -
        present)
    John Dennis, D.V.M., M.S., Comparative Medicine Graduate Program, antisense PKC-α (Penn State — 1994-
        1996)
        Current position: Clinical Veterinarian, Duke University School of Medicine
    Monica Embers, B.S. Microbiology and Immunology Graduate Program, Papillomavirus vaccine development
        (Penn State — 1998-present)
    Steven F. Goldberg, B.B.A., M.B.A.., Cell & Molecular Biology Program, Molecular biology of melanoma
        metastasis (Penn State — 1996-present)
    John Harms, B.S. Molecular Medicine Graduate Program, Molecular biology of melanoma metastasis (Penn

State — 1997-present)

Steven Harvey, D.V.M., M.S., Comparative Medicine Graduate Program, oral papillomavirus (Penn State — 1995-1997)

Louis P. Hodgson, B.S., Bioengineering Graduate Program, fluid dynamics in cell motility (Penn State — 1998-present)

Russell Hoover, B.S., Ph.D., Molecular Physiology Graduate Program, lung surfactant (Penn State — 1996-1998)

Karen LaPorte, B.S., Cell and Molecular Biology Graduate Program (Penn State — 2000-present)

Jennifer Maiale, B.S., Cell and Molecular Biology Graduate Program, DNA replication defects in cancer (Penn State — 1998-present)

William Meehan, B.S., M.S. Molecular Medicine and M.D./Ph.D. Graduate Programs, Molecular biology of melanoma metastasis (Penn State — 1997-present)

Jean Munnerlyn, M.S., Immunology, radiation biology (Upjohn- 1984-1986)

Kevin Nash, M.S., M.D./Ph.D. Graduate Program, KiSS1 clinical correlations (Penn State — 2000-present)

Jelena Pavlovic, B.S. Molecular Medicine and M.D./Ph.D. Graduate Programs, Lung surfactant proteins, (Penn State 1998 — present)

Bradley Rank, B.S., M.S. Bioengineering Graduate program, Fluid flow in cell extravasation (Penn State — 1998-2000)

Monica Richert, B.S., Ph.D., Cell & Molecular Biology Graduate Program, IGFBPs in breast development (Penn State — 1994-1998)

Margaret Slattery, B.S., Bioengineering Graduate Program, Motility of breast cancer cells (Penn State — 1999-present)

Malinda Stull, B.S., Cell & Molecular Biology Graduate Program, IGFBP in breast development (Penn State — 1998-present)

Mark Uhlik, B.S., Microbiology & Immunology Graduate Program, NfκB regulation (Penn State — 1997-present)

Jun You, M.S., Ph.D. Bioengineering Graduate Program, mechanisms of cell motility (Penn State — 1996-1998)

Postdoctoral and/or sabbatical

Robert Earhart, M.D., Ph.D., (Sabbatical) metastasis induction, growth factors (Upjohn)

Lingling Hou, D.V.M., Visiting Scientist, ocular melanoma metastasis (Penn State)
Current Position – Unknown

Cheol Kyu Hwang, Ph.D., Postdoctoral Fellow, genetics of breast cancer metastasis (Penn State)
Current Position – Postdoctoral Fellow, National Institutes of Health

Jeong-Hyung Lee, Ph.D., Postdoctoral Fellow, genetics of melanoma metastasis (Penn State)
Current Position – Investigator (Asst. Prof. equivalent) Korean Institute of Technology

Timothy O. Leonard, M.D., Ph.D., Postdoctoral Fellow, genetics of melanoma metastasis (Penn State)
Current Position – Resident (Penn State University, Department of Pathology)

James E. Malone, M.D., Fellow, metastasis-suppressors in head and neck cancer (Penn State)
Current Position – Chief Resident (Penn State University, Department of Surgery)

Mary E. Miele, Ph.D., Postdoctoral Fellow, genetics of melanoma metastasis (Penn State)
Current Position – Assistant Professor, University of Delaware

Shelia A. McClure, Ph.D., Visiting Scientist, cell surface glycoconjugates (Upjohn)
Current Position – Associate Professor, Spelman College

Carl T. McGary, M.D., Ph.D., Resident, Tumor-elicited neutrophils (Penn State)
Current Position – Assistant Professor, University of Rochester School of Medicine

Bruce E. Miller, Ph.D., Postdoctoral Fellow, tumor immunology, adhesion (Glaxo)
Current Position – Unknown

Toshiyuki Sakamaki, Ph.D. – Postdoctoral Fellow, breast cancer genetics (Penn State)

Lalita R. Shevde-Samant, Ph.D. – Postdoctoral Fellow, breast cancer genetics; head and neck cancer biology (Penn State)

Rajeev S. Samant, Ph.D., Postdoctoral Fellow, metastasis suppressor gene genetics (Penn State)

Jabed Seraj, M.D., Ph.D. Postdoctoral Fellow, breast cancer genetics (Penn State)
Current Position – Research Assistant, University of Virginia

Medical Student Trainees

Renee Allenbaugh, B.S. (Penn State — 1995-1996)

Dale Danglebren, B.S. (Penn State — 1996-2000)

Lisa L. Hamaker, B.S. (Penn State — 1999-2002)
Raymond Patterson, B.S. (Penn State — 1999-2003)

Patents

KiSS-1, a novel human melanoma metastasis-suppressor gene. U.S. Patent Application submitted October 11, 1996, Inventors: D.R. Welch and J.-H. Lee

KiSS-1, a novel human melanoma metastasis-suppressor gene. International Patent WO97/13778, April 17, 1997, Inventors: D.R. Welch and J.-H. Lee

BRMS1, a novel human breast carcinoma metastasis-suppressor gene. Patents Pending, May 1999, Inventors: D.R Welch, R.S. Samant, M.F. Verderame and M.J. Seraj

PROFESSIONAL SOCIETIES

Current:

American Cancer Society
    Board of Directors - Pennsylvania Division
        (1992 - present)
    Member - Carcinogenesis, Nutrition & The
        Environment Scientific Review Panel
American Association for Cancer Research
Metastasis Research Society
    Secretary/Treasurer (1998-2002)
National Cancer Institute Information Associates
American Association for the Advancement of Science
Sigma Xi Scientific Research Society
Women in Cancer Research

Past:

American Society for Cell Biology
U.T.-M.D. Anderson Associates
Lake Ontario Metastasis Research Group
Tissue Culture Association
    Texas Branch (officer 1981-1983)

CONSULTING:

Genzyme Corporation (1998-present) — Advise regarding product development and strategic planning for anti-invasive drug line.

Upstate Biotechnology Inc. (1996-1997) — Advise regarding product development and strategic planning for breast and prostate cancer product line.

SERVICE AND COMMITTEES:

Penn State University College of Medicine

2000	Reviewer, Four Diamonds Foundation (funds pediatric cancer research)
1999- present	Institutional Animal Care and Use Committee
1999- present	Co-chair, Penn State Cancer Center Education Committee
1998- present	Search Committee – John W. Kreider Chair, Jake Gittlen Cancer Research Institute
1998- present	Search Committee – Foreman Foundation Melanoma Biologist, Penn State Cancer Center
1997- present	Promotion and Tenure Review Committee - Department of Pathology
1997- present	Department of Pathology Seminar Committee
1997- present	Jake Gittlen Cancer Research Institute Seminar Committee
1995- present	Admissions interviewer - Medical School & Graduate Programs
1995- present	Teaching - Peer Review Evaluation Committee
1999	Promotion and Tenure Review Committee - Department of Comparative Medicine
1999	Chair, Graduate Education Committee, Department of Pathology
1999	Research Committee, Department of Pathology
1999	Organizing Committee – Penn State Cancer Center Annual Symposium
1999	Moderator – Penn State Cancer Center Symposium on Genomic Instability
1998	Organizing Committee – Penn State Cancer Center Annual Symposium
1998	Moderator – Biologic Concepts in Cancer with Clinical Implications for the Year 2000 and Beyond.

1997- 1999	Tumor Tissue Bank Use Committee
1996- 1997	Educational and Research Enterprise Committee
1995- 1996	Divisional Representative for Graduate Program in Molecular Medicine
1995- 1996	Rx2 - Redesign for Excellence-2, Committee to Improve Operational Efficiency of Hershey Medical Center (Design Team)
1992- 1996	Cultural and Ethnic Diversity Committee
1992- 1993	Employee Communications Task Force
1990- 1992	Experimental Pathology Search Committee
1992	Pennsylvania Society for Biomedical Research

Penn State University
| 1999- 2000 | Search Committee – Associate Professor of Biomolecular Nutrition and Carcinogenesis, College of Health and Human Development |

American Cancer Society
2000- 2002	Medical Director-at-Large – Pennsylvania Division
1999	Division Research Initiatives Blue Ribbon Panel II – National Office, Atlanta, GA
1999	Cancer Control 2015 Goals – National Office, Atlanta, GA
1999	Review Panel – Nutritional Supplements Research Proposal, Cambria Unit
1998- 2000	Media Spokesperson on Tobacco and Breast Cancer Related Issues - Commonwealth (PA) Division
1997- 2000	Chairman, Cancer Advocacy Advisory Committee - Commonwealth (PA) Division
1996- 1999	Medical Director-at-Large, Board of Directors - Commonwealth (PA) Division
1996- 1999	Member, Cancer Control Committee - Commonwealth (PA) Division
1994- 1996	Chairman, Breast Cancer Detection and Treatment Committee - Pennsylvania Division
1994- 1996	Member, Finance Committee, Pennsylvania Division
1994- 1995	Chairman, Detection and Treatment Committee - Pennsylvania Division
1992- 1996	Medical Director-at-Large: Board of Directors - Pennsylvania Division
1994- 1996	Administration Committee - Pennsylvania Division
1991	Professional Education Committee - Pennsylvania Division
1991- 1995	Public Relations Committee - Pennsylvania Division
1991- 1993	Public Education Committee - Pennsylvania Division
1984- 1988	Speakers Bureau, American Cancer Society - Michigan Division
1980- 1984	Speakers Bureau, American Cancer Society - Texas Division
1977- 1980	Speakers Bureau, American Cancer Society - California Division

Metastasis Research Society
1999- 2000	Scientific Organizing Committee – VIII International Congress of the Metastasis Research Society
2000	Chair and Keynote Speaker – VIII International Congress of the Metastasis Research Society
1998- 2002	Secretary/Treasurer
1998- 2002	Board of Directors
1998	Co-chair, Poster Discussion B VII International Congress of the Metastasis Research Society

American Association for Cancer Research
2000	Scientific Organizing Committee – AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics
2000	Gertrude Elion Award Selection Committee
1999	1999 Annual Meeting, Chairman - Cell & Tumor Biology Subcommittee of the Program Committee
1999	1999 Annual Meeting, Co-Chair - Minisymposium – Interactions of Metastatic Cells with their Environment – Genetic Implications
1995	1995 Annual Meeting, Program Committee, Subcommittee on Invasion, Metastasis, and Angiogenesis and Co-chairman - Minisymposium on Cancer Metastasis and Angiogenesis
1994	1994 Annual Meeting, Vice-chairman - Program Committee-Biology Section, and Co-chairman - Minisymposium on Cellular and Genetic Factors in Cancer Metastasis

Study Sections

1997- 2001	Member, Carcinogenesis, Nutrition & Environment Scientific Advisory Committee, American Cancer Society
2000	Member, Pathobiology I (PBY1), U.S. Army Research and Materiel Command Breast Cancer Program
	Chair, California Cancer Research Program — Biomedical Study Section C
	Member, California Breast Cancer Research Program – Basic Breast Cancer Study Section
	Member, American Institute for Cancer Research, Panel B Review Committee
	Reviewer, Veteran's Administration, Oncology Reviews
	Member, NIH/NCI Cancer Center Support Grant P30, University of Colorado Cancer Center
1999	Member, NIH/NCI Special Study Section, UO1, Early Detection Research Network – Clinical and Epidemiologic Centers (RFA-99-007).
	NCI/NIH Initial Review Group A Cancer Centers, *Ad Hoc*
	Member, NIH/NCI Cancer Center Support Grant P30, Vanderbilt University
	Member, Special Study Section, NIH/NCI, PO1 Melanoma Etiology, Progression and Therapy, Wistar Institute
1998	Member, NASA Biotechnology Review Panel
	Reviewer, The University of Liverpool Cancer Research Committee, North West Cancer Research Fund
	Reviewer, Breast Cancer Society of Canada 1998 Review Program
	Member, California Cancer Research Program — Biomedical Study Section B
	Member, Special Study Section, NIH/NCI, PO1 Melanoma Etiology, Progression and Therapy, Wistar Institute
	Reviewer, Special Study Section NIH/NCI, PO1 Melanoma Genetics (U. Pittsburgh)
	Member, American Institute for Cancer Research, Panel B Review Committee
	Member, Pathobiology I (PBY1), U.S. Army Research and Materiel Command Prostate Cancer Program
	Member, Pathobiology I (PBY1), U.S. Army Research and Materiel Command Breast Cancer Program
	Member, Cell Biology I (CBY1), U.S. Army Research and Materiel Command Breast Cancer Program
	Member, Panel Review B, American Institute for Cancer Research
	Reviewer, New Jersey Cancer Research Commission
1997	Ad Hoc Member, NCI/NIH Initial Review Group A Cancer Centers
	Reviewer, Special Study Section NIH/NCI, PO1 Melanoma Genetics (U. Pittsburgh)
	Reviewer, NIH/NCI, Cancer Center Support Grant P30 U. Minnesota
	Member, Pathobiology 1 (PBY-1), U.S. Army Research and Materiel Command Breast Cancer Res. Program
	Reviewer, New Jersey Cancer Research Commission
1996	Member, Pathobiology 1 (PBY-1), U.S. Army Research and Materiel Command Breast Cancer Res. Program
	Member, Molecular Genetics-3 (MBY-3), U.S. Army Research and Materiel Command Breast Cancer Res. Program
	Reviewer, NIH/NCI, Cancer Center Support Grant P30 Karmanos Cancer Center
	Member, Susan G. Komen Cancer Foundation for Breast Cancer Research
	Ad hoc Reviewer, Special Study Section for Oral Cancer P01 grants, NCI, NIH
	Ad hoc reviewer, Carcinogenesis and Nutrition Scientific Advisory Committee, American Cancer Society
	Reviewer, New Jersey Cancer Research Commission
1995	Member, Cell & Tissue Biology (CTB-1), U.S. Army Research and Materiel Command Breast Cancer Res. Program
	Ad hoc Reviewer, NAPBC Innovative Small Grant Program, Priority Area 4 (Panel B), NIH, NCI
	Reviewer, New Jersey Cancer Research Commission
1994	Member, Cell/Tissue Culture Review Panel, National Aeronautics and Space Administration

Ad hoc Reviewer, Carcinogenesis and Nutrition Scientific Advisory Committee, American Cancer Society

Member, Cell & Tissue Biology (CTB-2), U.S. Army Research and Materiel Command Breast Cancer Res. Program

1994 — Reviewer, New Jersey Cancer Research Commission

1988 - 1991 — Reviewer, Arizona Disease Control Research Commission - Neoplastic Diseases Review Panel

1990 — Reviewer, North Carolina Biotechnology Center

Local Community Service

1999-2000 — Treasurer – Community Evangelical Free Church of Harrisburg

1999-2001 — Board of Directors - Elder, Community Evangelical Free Church of Harrisburg

1999-present — Adult Teacher, Community Evangelical Free Church of Harrisburg

1999 — Development Team – Finance, Church Plant - Harrisburg Area Evangelical Free Church

1997 — Environmental Health Advisory Group, Pennsylvania State Department of Health - Office of the Physician General

1994 - present — Co-chair, Legg Mason 5K for Jake's Sake (fund raising event for Jake Gittlen Cancer Research Institute)

1996- 1997 — Search Committee for Assistant Pastor of Adult Ministries, Evangelical Free Church, Hershey, PA

1996- 1998 — Advisory committee for Adult Education/Congregational Life, Evangelical Free Church, Hershey, PA

1991- 1999 — Adult Teacher, Evangelical Free Church, Hershey, PA

1992- 1996 — Home Away from Home, Ministry for International Scholars, Hershey, PA

1988 — Chairman, Committee for Decency, Kalamazoo, Michigan

1993- 1996 — Biotechnology Vocational Curriculum Committee, Milton Hershey School

Other Professional Organizations

2000 — Department of Defense Breast Cancer Research Program Era of Hope Meeting, Bone Metastasis, Atlanta, GA (11 June)

International Centre for Genetic Engineering and Biotechnology, Co-organizer: Molecular Aspects of Growth Control and Apoptosis, Caracas, Venezuela (23 January - 1 February)

1999 — Joint Steering Committee on Public Policy, March 24, 1999 (Presentations to Pennsylvania Representatives and Senators to U.S. Congress)

1998 — National Foundation for Cancer Research, Co-chairman - Minisymposium on the use of genomics to identify novel diagnostics for cancer

1998 — Pennsylvania Congressional Liaison (PennCL) - lobbying for biomedical research interests (through FASEB)

1985 — Co-chairman: Brook Lodge Symposium "Cancer Metastasis: Experimental and Clinical Strategies"

1984 — Co-chairman: Organizing committee: 2nd Annual University of Texas Health Science Center at Houston - Graduate School of Biomedical Sciences Student Research Symposium

1983 — Program Committee: Spring Meeting of the Texas Branch - Tissue Culture Association, "Toxicology"

1983 — Program Committee & Session Chairman: Fall Meeting of the Texas Branch - Tissue Culture Association, "Cellular Interactions"

1983 — Chairman: University of Texas Health Science Center, Graduate School of Biomedical Sciences High School Teachers Workshop

1983 — Chairman: University of Texas Health Science Center, Graduate School of Biomedical Sciences Student Research Symposium

Glaxo Research Laboratories

1988- 1990 — Chemotherapy Discovery Group, Cell Adhesion (ICAM-1) Project Team, Drug Targeting Project Team, Topoisomerase I Inhibitor Project Team, Cancer Therapy Evaluation Group

The Upjohn Company

1988- 1989 — Cancer Research Seminar Committee

1988	Co-chairman: Ontario Metastasis Research Group - Fall Meeting
1987	Co-chairman: Kalamazoo College Senior Seminar Course
1984- 1988	Cancer Research Project Team, Minority Recruitment and Training Program, Cancer Treatment Project Team

EDITORIAL ACTIVITIES

Editorial Boards:

Anti-Cancer Drugs (2000-2003)

Regular Reviewer (approximate number of reviews per year)	Occasional Reviewer
American Journal of Pathology (2) *Cancer* (2-4) *Cancer Detection and Treatment* (3) *Cancer Research* (20) *Clinical Cancer Research* (6) *Clinical and Experimental Metastasis* (6-8) *Experimental Cell Research* (2) *International Journal of Cancer* (2) *Invasion and Metastasis* (1-2) *Journal of the National Cancer Institute* (3) *Nature Medicine* (1) *Oncogene* (2) *Science* (1)	*Anesthesia* *Biochemical and Biophysical Research Communications* *Biotechniques* *Cancer Letters* *Cell Growth and Differentiation* *Chemica-Biological Interactions* *CRC Press - Reviews* *European Journal of Cancer and Clinical Oncology* *Journal of Biological Chemistry* *Journal of Clinical Endocrinology and Metabolism* *Life Sciences* *Melanoma Research* *Molecular Carcinogenesis* *Molecular and Cellular Differentiation* *Nature* *Nature Genetics* *Oncology Research* *Proceedings of the National Academy of Sciences* *Toxicology and Applied Pharmacology* *Trends in Biochemical Sciences*

PUBLICATIONS
– Danny R. Welch, Ph.D. –

EDITOR

CANCER METASTASIS: EXPERIMENTAL AND CLINICAL STRATEGIES, **D.R. Welch**, B.K. Bhuyan and L.A. Liotta, eds., Alan R. Liss, Inc., 1986

LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY — METASTASIS, Burger, M.M., Rusciano, D. and **Welch, D.R.** (In press)

PUBLICATIONS IN PEER-REVIEWED JOURNALS

1. Neri, A., **Welch, D.R.**, Kawaguchi, T. & Nicolson, G.L. Development and biologic properties of malignant cells and clones of a spontaneously metastasizing rat mammary adenocarcinoma. *Journal of the National Cancer Institute* (1982) <u>68</u>: 507-517.

2. **Welch, D.R.**, Milas, L., Tomasovic, S.P., & Nicolson, G.L. Heterogeneous response and clonal drift of metastatic 13762NF mammary adenocarcinoma clones to gamma radiation *in vitro, Cancer Research* (1983) <u>43</u>: 6-10.

3. **Welch, D.R.**, Neri, A. & Nicolson, G.L. Comparison of 'spontaneous' and 'experimental' metastasis using rat 13762NF mammary adenocarcinoma cell clones. *Invasion and Metastasis* (1983) <u>3</u>:65-80.

4. **Welch, D.R.** & Nicolson, G.L. Phenotypic drift and heterogeneity in response of metastatic mammary adenocarcinoma cell clones to Adriamycin, 5-fluoro-2'-deoxyuridine and Methotrexate *in vitro. Clinical and Experimental Metastasis* (1983) <u>1</u>:317-325.

5. Pearce, V., Pathak, S., Mellard, D., **Welch, D.R.** & Nicolson, G.L. Chromosome and DNA analyses of rat 13762NF mammary adenocarcinoma cell lines and clones of different metastatic potentials. *Clinical and Experimental Metastasis* (1984) <u>2</u>:271-286.

6. **Welch, D.R.**, Krizman, D.B. & Nicolson, G.L. Multiple phenotypic divergence of mammary adenocarcinoma cell clones. I. In vitro and in vivo properties. *Clinical and Experimental Metastasis* (1984) <u>2</u>:333-355.

7. **Welch, D.R.**, Evans, D.P., Tomasovic, S.P., Milas, L. & Nicolson, G.L. Multiple phenotypic divergence of mammary adenocarcinoma cell clones. II. Sensitivity to radiation, hyperthermia and FUdR. *Clinical and Experimental Metastasis* (1984) <u>2</u>:357-371.

8. Nicolson, G.L., Dulski, K., Basson, C.T. & **Welch, D.R.** Preferential organ attachment and invasion in vitro by B16 melanoma cells selected for differing metastatic colonization and invasive properties. *Invasion and Metastasis* (1985) <u>5</u>:144-158.

9. **Welch, D.R.** & Tomasovic, S.P. Implications of tumor progression on clinical oncology. *Clinical and Experimental Metastasis* (1985) <u>3</u>:151-188.

10. Tomasovic, S.P. & **Welch, D.R.** Heat stress proteins and experimental cancer metastasis. *International Journal of Hyperthermia* (1986) <u>2</u>:253-266.

11. Nakajima, M., **Welch, D.R.**, Belloni, P.N. & Nicolson, G.L. Degradation of basement membrane type IV collagen and lung subendothelial matrix by rat mammary adenocarcinoma cell clones of differing metastatic potentials. *Cancer Research* (1987) <u>47</u>:4869-4876.

12. Tomasovic, S.P., Armour, E.P., North, S.M. & **Welch, D.R.** Rat mammary adenocarcinoma heat-stress proteins in vivo. *International Journal of Hyperthermia* (1987) <u>3</u>:467-473.

13. **Welch, D.R.** Biologic considerations for drug targeting in cancer patients. *Cancer Treatment Reviews* (1987) <u>14</u>:351-358.

14. Nicolson, G.L., Lembo, T.M. & **Welch, D.R.** Growth of rat mammary adenocarcinoma cells in semisolid

clonogenic medium not correlated with spontaneous metastatic behavior: Heterogeneity in the metastatic, antigenic, enzymatic and drug sensitivity properties of cells from different sized colonies. *Cancer Research* (1988) <u>48</u>:399-404.

15. **Welch, D.R.**, Aeed, P.A. & Estrada, J. Development and characterization of a rat model for locally recurring mammary tumors: Sensitivities to 5-fluoro-2'-deoxyuridine, Adriamycin and X-irradiation. *Cancer Research* (1988) <u>48</u>:4549-4554.

16. Aeed, P.A., Nakajima, M. & **Welch, D.R.** The role of polymorphonuclear leukocytes (PMN) on the growth and metastatic potential of 13762NF mammary adenocarcinoma cells. *International Journal of Cancer* (1988) <u>42</u>:748-759.

17. Aeed, P.A. & **Welch, D.R.** Sensitivity of locally recurrent rat mammary tumour cell lines to syngeneic polymorphonuclear cell, macrophage and natural killer cell cytolysis. *British Journal of Cancer* (1988) <u>58</u>:746-752.

18. Estrada, J., Freeman, D.L., Aeed, P.A. & **Welch, D.R.** Experimental model for locally recurring mammary tumors: Development, morphology, karyotype, growth kinetics and experimental metastatic potential. *Cancer* (1989) <u>63</u>:1353-1362.

19. **Welch, D.R.**, Lobl, T.J., Seftor, E.A., Wack, P.J., Aeed, P.A., Yohem, K.H., Seftor, R.E.B. & Hendrix, M.J.C. Use of the membrane invasion culture system (MICS) as a screen for anti-invasive agents. *International Journal of Cancer* (1989) <u>43</u>:449-457.

20. **Welch, D.R.**, Schissel, D.J., Howrey, R.P. & Aeed, P.A. Tumor-elicited polymorphonuclear cells, in contrast to 'normal' circulating polymorphonuclear cells, stimulate invasive and metastatic potentials of rat mammary adenocarcinoma cells. *Proceedings of the National Academy of Science (USA)* (1989) <u>86</u>:5859-5863.

21. Scieszka, J.F., Aeed, P.A., **Welch, D.R.** & Cho, M.J. Neutrophil-mediated transfer of polar substances from liposomes to mammary tumor cells in vitro. *International Journal of Pharmaceutics* (1989) <u>5319</u>:167-173.

22. Hendrix, M.J.C., Seftor, E.A., Seftor, R.E.B., Misiorowski, R.L., Saba, P.Z., Sundareshan, P. & **Welch, D.R.** Comparison of tumor cell invasion assays: human amnion versus reconstituted basement membrane barriers. *Invasion and Metastasis* (1989) <u>9</u>:278-297.

23. Bevacqua, S.J., **Welch, D.R.**, Diez de Piños, S.M., Shapiro, S.A., Johnston, M.G., Witte, M.H., Leong, S.P.L., Dorrance, T.L., Leibovitz, A. & Hendrix, M.J.C. Quantitation of human melanoma, carcinoma and sarcoma tumor cell adhesion to lymphatic endothelium. *Lymphology* (1990) <u>23</u>:4-14.

24. **Welch, D.R.**, Fabra, A. & Nakajima, M. Transforming growth factor-beta stimulates mammary adenocarcinoma cell invasion and metastatic potential. *Proceedings of the National Academy of Science (USA)* (1990) <u>87</u>:7678-7682.

25. **Welch, D.R.**, McClure, S.A., Aeed, P.A., Bahner, M.J. & Adams, L.D. Tumor progression- and metastasis-associated proteins identified using a model of locally recurrent rat mammary adenocarcinomas. *Clinical and Experimental Metastasis* (1990) <u>6</u>:533-551.

26. **Welch, D.R.**, Bisi, J.E., Miller, B.E., Conaway, D., Seftor, E.A., Yohem, K.H., Gilmore, L.B., Seftor, R.E.B., Nakajima, M. & Hendrix, M.J.C. Characterization of a highly invasive and spontaneously metastatic human malignant melanoma cell line. *International Journal of Cancer* (1991) <u>47</u>:227-237.

27. Seftor, R.E.B., Seftor, E.A., Grimes, W.J., **Welch, D.R.** & Hendrix, M.J.C. Human melanoma cell invasion is inhibited in vitro by swainsonine and deoxymannojirimycin with a concomitant decrease in collagenase IV expression. *Melanoma Research* (1991) <u>1</u>:43-54.

28. Hendrix, M.J.C., Seftor, E.A., Chu, Y.-W., Seftor, R.E.B., Nagle, R.B., McDaniel, K.M., Leong, S.P.L., Yohem, K.H., Leibovitz, A.M., Meyskens, F.L.,Jr., Conaway, D.H., **Welch, D.R.**, Liotta, L.A. & Stetler-Stevenson, W.G. Co-expression of vimentin and cytokeratins by human melanoma tumor cells: correlation with invasive and metastatic potential. *Journal of the National Cancer Institute* (1992) <u>84</u>:165-174.

29. Yohem, K.H., Clothier, J.L., Montague, S.L., Geary, R.J., Winters, A.L., Hendrix, M.J.C. & **Welch, D.R.** Inhibition of tumor cell invasion by verapamil. *Pigment Cell Research* (1992) 4:225-233.

30. Harper, D.E. & **Welch, D.R.** Isolation, purification, synthesis and antiinvasive/antimetastatic activity of U-77863 and U-77864 from *Streptomyces griseoluteus*, Strain WS6724. *Journal of Antibiotics* (1992) **45**:1827-1836.

31. **Welch, D.R.**, Harper, D.E., & Yohem, K.H. U-77,863: A novel cinnanamide isolated from *Streptomyces griseoluteus* that inhibits tumor cell invasion and metastasis. *Clinical and Experimental Metastasis.* (1993) **11**: 201-212.

32. Nakajima, M., **Welch, D.R.**, Wynn, D.M., Tsuruo, T. & Nicolson, G.L. Serum and plasma 92-kDa gelatinase activities correlate with spontaneous lung metastasis formation of rat 13762NF mammary adenocarcinoma. *Cancer Research* (1993) **53**: 5802-5807.

33. **Welch, D.R.**, Chen, P., M.E. Miele., Bower, J.M., McGary, C.T., Stanbridge, E.J. & Weissman, B.E. Microcell-mediated transfer of chromosome 6 into metastatic human C8161 melanoma cells suppresses metastasis, but not inhibit tumorigenicity. *Oncogene* (1994) **9**: 255-262.

34. Suit, H., Allam, A., Allalunis-Turner, J., Brock, W., Girinsky, T., Hill, S., Hunter, N., Milas, L., Pearcey, R., Peters, L., **Welch, D.R.**, West, C., & Efird, J. Is tumor cell radiation resistance correlated with metastatic ability? *Cancer Research* (1994) **54**: 1736-1741.

35. Miele, M.E., Bennett, C.F., Miller, B.E. & **Welch, D.R.** Enhanced metastatic ability of TNF-α-treated malignant melanoma cells is reduced by ICAM-1 (CD54) antisense oligonucleotides. *Experimental Cell Research* (1994) **214**: 231-241.

36. **Welch, D.R.**, Aeed, P.A., Earhart, R.H & McClure, S.A. Evidence for paracrine regulation of experimental metastasis in 13762NF rat mammary adenocarcinoma cell clones. *Anticancer Research* (1994) **14**: 1743-1752.

37. Jiang, H., Lin, J., Herlyn, M., Kerbel, R.S., Weissman, B.E. , **Welch, D.R.** & Fisher, P.B. mda-6, WAF/CIP1, is a melanoma differentiation-associated gene displaying differential expression during growth, differentiation and progression in human melanoma cells. *Oncogene* (1995) **10**: 1855-1864.

38. You, J., Miele, M.E., Dong, C. & **Welch, D.R.** Suppression of human melanoma metastasis by introduction of chromosome 6 may be partially due to inhibition of motility, but not to inhibition of invasion. *Biochemical and Biophysical Research Communications* (1995) **208**: 476-484.

39. Miele, M.E., McGary, C.T. and **Welch, D.R.** SOD2 (MnSOD) does not suppress tumorigenicity or metastasis of human C8161 melanoma cells. *Anticancer Research* (1995) **15**: 2065-2070.

40. McGary, C.T., Miele, M.E., **Welch, D.R.** Highly metastatic 13762NF rat mammary adenocarcinoma clones secrete IL-3 or GM-CSF-like activity that is apparently responsible for neutrophilia response. *American Journal of Pathology* (1995) **147**: 1668-1681.

41. Rieber, M.S., **Welch, D.R.**, Miele, M.E. and Rieber, M.. p53-independent increase in $p21^{WAF1}$ and reciprocal down-regulation of cyclin A and proliferating cell nuclear antigen in bromodeoxyuridine-mediated growth arrest of human melanoma cells. *Cell Growth and Differentiation* (1996) **7**: 197-202.

42. Phillips, K.*, **Welch, D.R.***, Miele, M.E., Lee, J.-H., Wei., L.L., and Weissman, B.E. Suppression of MDA-MB-435 breast carcinoma cell metastasis following the introduction of human chromosome 11. *Cancer Research* (1996) **56**: 1222-1227. * *Contributed equally to this work.*

43. **Welch, D.R.** and Rieber, M., Is $p21^{mda6/WAF1}$ a malignant melanoma metastasis-suppressor gene? *Molecular and Cellular Differentiation*, (1996), **4**: 91-111.

44. Miele, M.E., Lee, J.-H., Coleman, A., Robertson, G.,McGary, C.T., Lugo, T.G. and **Welch, D.R.** Introduction of human chromosome 6 and chromosome 1 into human melanoma cell line MelJuSo suppresses metastasis. *Molecular Carcinogenesis* (1996) **15**: 284-299.

45. **Welch, D.R.** (Invited editorial) Secrecy in Research: Is it hurting progress against cancer? *Oncology Times*

(1996) **18**: 37-38. [The editorial also includes excerpts from several other scientists and physicians].

46. Lee, J.-H., Miele, M.E., Hicks, D.J., Phillips, K.K., Trent, J.M., Weissman, B.E. and **Welch, D.R.** (1996) KiSS-1, A novel malignant melanoma metastasis-suppressor genes identified in chromosome 6-malignant melanoma microcell hybrids. *Journal of the National Cancer Institute* **88**: 1731-1737.

47. Miele, M.E., de la Rosa, A., Lee, J.-H., Hicks, D.J., Dennis, J.U., Steeg, P.S. and **Welch, D.R.** (1997) NME1 (Nm23-H1) levels rise in metastasis-suppressed chromosome 6/human melanoma hybrids, but transfection with NME1 does not completely suppress metastasis. *Clinical and Experimental Metastasis* **15**: 259-265.

48. McGary, C.T., Pan, Y.-c., Neri, A., Guntrum, W.D. and **Welch, D.R.** (1997) Tumor-elicited, metastasis-enhancing neutrophils, but not circulating or phorbol ester-activated PMN, express high levels of MRP14. *Anticancer Research* **17**: 1-6.

49. **Welch, D.R.** Technical considerations when studying cancer metastasis *in vivo*. (1997) *Clinical and Experimental Metastasis* **15**(3): 272-306.

50. Lee, J.-H. and **Welch, D.R.** (1997) Identification of highly expressed genes in metastasis-suppressed chromosome 6/human malignant melanoma hybrid cells using subtractive hybridization and differential display. *International Journal of Cancer* **71**: 1035-1044

51. Lee, J-H. and **Welch, D.R.** (1997) Suppression of metastasis in human breast carcinoma MDA-MB-435 cells following transfection with the metastasis-suppressor gene, *KISS-1*. *Cancer Research* **57**: 2284-2287.

52. Yang, X., **Welch, D.R.**, Phillips, K.K., Weissman, B.E., Wei, L.L. (1997) KAI-1, a putative marker for metastatic potential in human breast cancer. *Cancer Letters* **119**: 149-155.

53. Phillips, K.K., White, A.E., Hicks, D.J., **Welch, D.R.**, Barrett, J.C., Wei, L.L. and Weissman, B.E. (1998) Suppression of metastasis in the MDA-MB-435 model system correlates with increased expression of KAI-1 protein. *Molecular Carcinogenesis* **21**: 111-120.

54. **Welch, D.R.** and Goldberg, S.F. (1997) Molecular mechanisms controlling human melanoma progression and metastasis. *Pathobiology* **65**: 311-330

55. **Welch, D.R.** and Wei, L.L. (1998) Molecular control of breast cancer progression and metastasis. *Endocrine Related Cancers* **5**: 155-196.

56. Dennis, J.U., Dean, N.M., Bennett, C.F., Griffith, J.W., Lang, C.M. and **Welch, D.R.** (1998) Human melanoma metastasis is inhibited following *ex vivo* treatment with an antisense oligonucleotide to protein kinase C-α. *Cancer Letters* **128**: 65-70.

57. Robertson, G.P., Furnari, F.B., Miele, M.E., Glendening, M.J., Arden, K., **Welch, D.R.**, Fountain, J.W., Lugo, T.G., Huang, S., Cavenee, W.K. (1998) An in vitro chromosome breakage-loss of heterozygosity model provides functional evidence implicating PTEN/MMAC1 in melanoma tumorigenesis. *Proc. Natl. Acad. Sciences* **95**: 9418-9423.

58. Goldberg, S.F. and **Welch, D.R.** (1998) KiSS-1 – A suppressor of cancer metastasis. *Jikken Igaku (Experimental Medicine)* (Article translated into Japanese) **16**: 37-46.

59. West, A., Vjota, P. **Welch, D.R.** and Weissman, B.E. (1998) Chromosome localization and genomic structure of the KiSS-1 metastasis suppressor gene. *Genomics* **54**: 145-148.

60. **Welch, D.R.**, Harms, J.F., Goldberg, S.F., Meehan, W.J., Seraj, M.J., Leonard, T.O., Samant, R.S., Miele, M.E., Lee, J.-H. and Hicks, D.J. (1999) Identifying and characterizing metastasis-suppressor genes in human cancer. *Biological Approaches to Cancer Therapy* **1**: 32-38.

61. Meehan, W.J., Spencer, J.P.E., Rannels, D.E., **Welch, D.R.**, Knobbe, E.T., and Ostrander, G.K. (1999) Hydrogen peroxide induces oxidative damage in rat type II pulmonary epithelial cells. *Environmental and Molecular Mutagenesis* **33**: 273-278.

62. Kiley, S.C., Clark, K.J., Duddy, S.K., **Welch, D.R.** and Jaken, S. (1999) Protein kinase C δ involvement in mammary tumor cell metastasis. *Cancer Research* **59**: 3230-3238.

63. Kiley, S.C., Clark, K.J., Duddy,S.K., **Welch, D.R.** and Jaken, S. (1999) Increased protein kinase C-δ in mammary tumor cells: relationship to transformation and metastatic progression. *Oncogene* 18: 6748-6757.

64. **Welch, D.R.** and Rinker-Schaeffer, C.W. (1999) What defines a useful marker of metastasis in human cancer? [Invited editorial]. *Journal of the National Cancer Institute* 91: 1351-1353.

65. Miele, M.E., Jewett, M.D., Goldberg, S.F., Hyatt, D.L., Morelli, C., Gualandi, F., Rimessi, P., Hicks, D.J., Weissman, B.E., Barbanti-Brodano, G., **Welch, D.R.** (2000) A human melanoma metastasis-suppressor locus maps to 6q16.3-q23. *International Journal of Cancer* 86: 524-528.

66. Goldberg, S.F.*, Harms, J.F.*, Quon, K. and **Welch, D.R.** (1999) Metastasis-suppressed C8161 melanoma cells arrest in lung but fail to proliferate. *Clinical and Experimental Metastasis* 17: 601-607. * *Contributed equally to this work.*

67. **Welch, D.R.**, Sakamaki, T., Pioquinto, R., Leonard, T.O., Goldberg, S.F., Hon, Q., Erikson, R.L., Rieber, M., Strasberg-Rieber, M., Hicks, D.J., Bonventre, J.V., Alessandrini, A. (2000) Transfection of constitutively active Mek1 confers tumorigenic and metastatic potentials to NIH3T3 cells. *Cancer Research* 60: 1552-1556.

68. **Welch, D.R.**, Steeg, P.S., and Rinker-Schaeffer, C.W., (2000) Genetic regulation of human breast carcinoma metastasis. *Biology of Breast Cancer Metastasis* (Current Sciences Ltd.) (In press).

69. Seraj, M.J.*, Samant, R.S.*, Verderame, M.F., **Welch, D.R.** (2000) Functional evidence for a novel human breast carcinoma metastasis suppressor, *BRMS1*, encoded at chromosome 11q13 * *Contributed equally to this work. Cancer Research* 60: 2764-2769.

70. Yoshida, B., **Welch, D.R.** Rinker-Schaeffer, C.W. Metastasis suppressor genes. *Journal of the National Cancer Institute* (In press)

71. Rinker-Schaeffer, C.W., **Welch, D.R.**, Sokoloff, M. (2000) Defining the biological role of genes that regulate prostate cancer metastasis. *Current Opinion in Urology* (In press).

• Rieber, M., **Welch, D.R.**, Strasberg-Rieber, M. Differential gene expression associated with suppression of metastatic melanoma cell spreading and proliferation on adhesion-restrictive substrates. (Submitted)

• Samant, R.S.*, Sakamaki, T.*, Seraj, M.J.*, Shevde, L.A., Meehan, W.J., Harms, J.F., Budgeon, L., Leonard, T.O., Winter, C.R., Christensen, N.D., Verderame, M.F., and **Welch, D.R.** Characterization of BRMS1 suppression of human breast carcinoma metastasis. (In preparation) * *Contributed equally to this work.*

• Samant, R.S., Debies, M.T., Seraj, M.J., Verderame, M.F., and **Welch, D.R.** Genomic structure of the human breast carcinoma metastasis suppressor gene, BRMS1. (In preparation)

• Samant, R.S., Debies, M.T. and **Welch, D.R.** Cloning and partial characterization of *brms1*, the murine homolog of the human breast carcinoma metastasis suppressor gene, *BRMS1*. (In preparation)

• Pethiyagoda, C.L., **Welch, D.R.**, Fleming, T.P. Dipeptidyl peptidase IV (DPPIV) inhibits cellular invasion of melanoma cells. (Submitted).

• **Welch, D.R.**, Miele, M.E., Chen, P., Fleming, T.P., and Weissman, B.E. Suppression of metastasis in the ocular melanoma, OM431, and the cutaneous melanoma, A375M, following introduction of human chromosome 6 by microcell-mediated chromosome transfer. (In preparation).

BOOKS AND CHAPTERS

1. Ph.D. Dissertation: Tumor Progression: Analysis of the Instability of the Metastatic Phenotype, Sensitivity to Radiation and Chemotherapy

2. Nicolson, G.L., Steck, P.A., **Welch, D.R.** & Lembo, T.M. Heterogeneity and instability of phenotypic and metastatic properties of local tumor- and metastasis-derived clones of a mammary adenocarcinoma. In: *Understanding Breast Cancer: Clinical and laboratory concepts*, Editors: Rich, M.A., Hager, J.C. & Furmanski, P. Marcel Dekker, Inc., New York (1983) pp.145-166.

3. **Welch, D.R.**, Evans, D.P., Tomasovic, S.P., Krizman, D.B., Milas, L. & Nicolson, G.L. Simultaneous and independent drift of multiple phenotypes in mammary adenocarcinoma cell clones. In: *Treatment of Metastasis: Problems and Prospects*, Editors: Hellmann, K. and Eccles, S.A. Taylor and Francis, Ltd., London (1984) pp 239-242.

4. **Welch, D.R.** Discussion of the suitability, availability and requirements for *in vivo* and *in vitro* models of metastasis. In: *Cancer Metastasis: Experimental and Clinical Strategies*, Editors: Welch, D.R., Bhuyan, B.K. & Liotta, L.A. Alan R. Liss, New York (1986) pp. 123-150.

5. Nakajima, M., **Welch, D.R.**, Irimura, T. & Nicolson, G.L. Basement membrane degradative enzymes as possible markers of tumor metastasis. In: *Cancer Metastasis: Experimental and Clinical Strategies*, Editors: Welch, D.R., Bhuyan, B.K. & Liotta, L.A. Alan R. Liss, New York (1986) pp. 113-122.

6. **Welch, D.R.** Factors involved in the development and maintenance of tumor heterogeneity. In: *Carcinogenesis and Dietary Fat*, Editor: Abraham, S. Kluwer Academic Publishers, Boston (1989) pp.279-301.

7. **Welch, D.R.** & Nakajima, M. Roles of TGF-β and FGF in cancer metastasis. In: *Mechanisms of Drug Resistance in Oncology*, Editor: Teicher, B.A. Marcel Dekker, Inc. New York (1993) pp. 561-581.

8. **Welch, D.R.** In vivo cancer metastasis assays. In: *Laboratory Techniques in Biochemistry and Molecular Biology*, Editors: Burger, M.M., Rusciano, D. and Welch, D.R. (In press)

9. **Welch, D.R.** Metastasis suppressor genes. In: *Cancer Research: an encyclopedic reference*, Editor: Schwab, M., Springer, Berlin (2000) (In press)

10. Goldberg, S.F. and **Welch, D.R.** KiSS1. In: *Cancer Research: an encyclopedic reference*, Editor: Schwab, M., Springer, Berlin (2000) (In press)

11. Samant, R.S. and **Welch, D.R.** BRMS1. In: *Cancer Research: an encyclopedic reference*, Editor: Schwab, M., Springer, Berlin (2000) (In press)

ABSTRACTS IN PUBLISHED PROCEEDINGS

Nicolson, G.L., Neri, A., **Welch, D.R** & Kawaguchi, T. Biological properties of malignant sublines and clones of a spontaneously metastasizing mammary adenocarcinoma. *Breast Cancer Research and Treatment* (1981) 1: 174.

Welch, D.R. & Nicolson, G.L. Heterogeneity and instability of metastatic tumor cell response to radiation and chemotherapy *in vitro*. (1983) Graduate Student Research Symposium

Welch, D.R. & Nicolson, G.L. Phenotypic drift and heterogeneity in response of metastatic mammary adenocarcinoma cell clones to Adriamycin, 5-fluoro-2'-deoxyuridine and Methotrexate treatment in vitro. *Clinical and Experimental Metastasis* (1983) 1:317-325.

Welch, D.R. & Nicolson, G.L. Instability of metastatic mammary tumor cell sensitivity to ionizing radiation. *Proceedings of the American Association for Cancer Research* (1984) 25: 62.

Nakajima, M. Custead, S.E., **Welch, D.R.** & Nicolson, G.L. Type IV collagenolysis: relation to metastatic properties of rat 13762NF mammary adenocarcinoma metastatic cell clones. *Proceedings of the American Association for Cancer Research* (1984) 25: 62.

Welch, D.R., Evans, D.P., Tomasovic, S.P., Krizman, D., Milas, L. & Nicolson, G.L. Simultaneous and independent drift of multiple phenotypes in mammary adenocarcinoma cell clones. *Treatment of Metastasis: Problems and Prospects*, pp. 4-10.

Nakajima, M., **Welch, D.R.**, Custead, S.E., Lembo, T.M. & Nicolson, G.L. Type IV collagenolysis by metastatic tumor and endothelial cells. *Proceedings of the American Society for Cell Biology* (1984) 99: 88a.

Nicolson, G.L., Dulski, K., & **Welch, D.R.** Preferential organ adhesion and invasion by B16 melanoma cells selected for differing metastatic colonization properties. Proceedings of the American Association for Cancer Research (1985) 26:195.

Tomasovic, S.O., Updyke, T.V., Rodriguez, L., Armour, E.P., & **Welch, D.R.** Hyperthermic stress proteins of metastatic tumor cell subpopulations and syngeneic normal cells. Cold Spring Harbor Laboratory Conference on

Heat Shock Induction of Proteins (1986).

Welch, D.R., Aeed, P.A., & Estrada, J. Characterization of locally recurring mammary tumors: Sensitivities to FUdR and Adriamycin. Proceedings of the American Association for Cancer Research (1986) 27:241.

Estrada, J., Freeman, D.H., & **Welch, D.R.** Characterization of locally recurring mammary tumors: differences in growth kinetics and metastatic potential. Proceedings of the American Association for Cancer Research (1986) 27:263.

Tomasovic, S.P. & **Welch, D.R.** Experimental cancer metastasis following heat and arsenite stress protein synthesis. Proceedings of the Union Internationale du Cancer (1986).

Welch, D.R., Aeed, P.A., Freeman, D.H., & Estrada, J. Locally recurrent mammary tumors exhibit sensitivities to chemotherapy agents different from primary tumors. Treatment of Metastasis Clinical and Experimental Metastasis (1986) 4:312.

Welch, D.R. & Tomasovic, S.P. Experimental mammary tumor metastasis following heat and arsenite stress protein synthesis. Treatment of Metastasis (1986).

Lobl, T.J., Hsi, E., Nishizawa, E.E., & **Welch, D.R.** Synthesis of a series of RGD-containing analogs derived from proteins: results from the human platelet aggregation assay and an experimental metastasis assay. Tenth American Peptide Symposium (1987).

Welch, D.R. Biologic considerations for drug delivery and drug targeting in cancer. Anticancer Drugs - Therapeutic Index Improvement by Toxicity Reduction. Anticancer Drug Design (1987) 2:98.

Welch, D.R. & Munnerlyn, J. Pretreatment of 13762NF mammary adenocarcinoma cells with 5-azacytidine reduces repair of sublethal (Elkind) radiation damage. Journal of Cell Biology (1987) 105:1379.

Aeed, P.A. & **Welch, D.R.** The influence of polymorphonuclear cells (PMN) on tumor growth and metastasis of 13762NF mammary adenocarcinoma cells. Journal of Cell Biology (1987) 105:1015.

Bevacqua, S., dePiños, S., Shapiro, S., **Welch, D.R.**, Johnson, M., Witte, M., Leong, S., Leibovitz, A., & Hendrix, M. Preferential tumor cell attachment to lymphatic endothelium. American Federation for Clinical Research (1988).

Welch, D.R. & Aeed, P.A. Effect of confluence on experimental metastatic potential of 13762NF mammary adenocarcinoma cells. Proceedings of the American Association for Cancer Research (1988) 29:68.

Aeed, P.A. & **Welch, D.R.** Neutrophilia from 13762NF mammary adenocarcinoma cell lines correlates with metastatic potential. Proceedings of the American Association for Cancer Research (1988) 29:67.

Adams, L.D., Aeed, P.A., & **Welch, D.R.** Characterization of locally recurrent mammary tumor cells by 2-D electrophoresis and automated densitometry. Intl. Electrophoresis Meeting (1988).

Welch, D.R., Earhart, R.H., Aeed, P.A., Howrey, R., Schissel, D.J., & Hendrix, M.J.C. Experimental metastatic potential of rat mammary tumor cells is modulated by factors secreted by tumor cells. Proceedings of the American Association for Cancer Research (1989) 30: 352.

Welch, D.R., Schissel, D.J., & Aeed, P.A. Syngeneic polymorphonuclear leukocytes (PMN) from 13762NF mammary tumor bearing rats stimulate *in vitro* tumor cell invasion and *in vivo* experimental metastatic potential. Critical Considerations for Tumor Progression and Metastasis (1989).

Ward, S.M., Miller, B.E., **Welch, D.R.**, Myers, D.L., & Blair, P.C. Effects of anti-rat neutrophil antibody on circulating PMN levels. Manual leukocyte differential vs Technicon H-1. Society of Toxicology (1990).

Welch, D.R., Bisi, J.E., Harper, D.E., Yohem, K.H., Seftor, E.A., & Hendrix, M.J.C. Identification of a novel inhibitor of tumor cell invasion and metastasis. Proceedings of the American Association for Cancer Research (1990) 31:2437.

Gilmore, L.B. & **Welch, D.R.** TGF-β stimulates lung colonization by rat 13762NF mammary adenocarcinoma clone MTLn3. Proceedings of the American Association for Cancer Research (1990) 31:376.

Miller, B.E. & **Welch, D.R.** Cytokine modulation of intercellular adhesion molecule-1 surface expression on human melanoma cells: correlation with adhesion of peripheral blood leukocytes. Proceedings of the American Association of Cancer Research (1990) 31:353.

Seftor, E.A., Nakajima, M., Stetler-Stevenson, W., **Welch, D.R.**, Liotta, L.A., & Hendrix, M.J.C. Selection for increased invasive potential *in vitro* correlates with increased protease expression and metastatic potential. Proceedings of the American Association for Cancer Research (1990) 31:430.

Yohem, K.H., Clothier, J.L., Montague, S.L., Geary, R.J., Winters, A.L., Hendrix, M.J.C., & **Welch, D.R.** Modulation of human melanoma cell invasion by calcium channel blockers. Proceedings of the American Association for Cancer Research (1990) 31:484.

Meade-Tollin, L.C., Yohem, K., **Welch, D.R.**, Pipes, B., Geary, R., Shimizu, N., & Hendrix, M.J.C. DNA breaks correlate with topoisomerase expression in cultured human melanoma cells. Proceedings of the American Association for Cancer Research (1990) 31:623.

Welch, D.R., Bisi, J.E., Miller, B.E., Gilmore, L.B., Conaway, D.H., Yohem, K.H., Seftor, E.A., Nakajima, M., & Hendrix, M.J.C. Characterization of C8161, a highly invasive and spontaneously metastatic human malignant melanoma cell line. Clinical and Experimental Metastasis (1990) 8:37.

Welch, D.R., Bisi, J.E., Yohem, K.H., & Hendrix, M.J.C. Inhibitors of protein kinase activation and Ca^{+2} mobilization lower human melanoma cell invasion and metastatic potentials. Clinical and Experimental Metastasis (1990) 8:167.

Miller, B.E. & **Welch, D.R.** Intercellular Adhesion Molecule-1 (ICAM-1) expression by human melanoma cells: association with leukocyte aggregation and metastatic potential. Clinical and Experimental Metastasis (1990) 8:80.

Hall, I.J., **Welch, D.R.**, & Tlsty, T.D. Gene amplification in metastatic rat mammary adenocarcinoma cell lines. Molecular Mechanism of Carcinogenesis in Human and Rodents (1991), Sept. 8-11, NIEHS.

Welch, D.R. Paracrine factors regulating tumor metastasis. Proceedings of the American Association for Cancer Research (1992) 33: 330.

Miele, M.E., Bennett, C.F., Freiberg, A.S. and **Welch, D.R.** Enhancement of human melanoma metastasis by TNF-α is inhibited by antisense ICAM-1 oligonucleotides. Proceedings of the American Association for Cancer Research (1993) 34: 381 [*winner of Upjohn Travel Award*]

Hall, I.J., **Welch, D.R.**, Tlsty, T.D. Genomic instability does not increase as tumor cells progress toward malignancy. Loss of Genomic Instability in Neoplasia (1993) 17: 25.

Hall, I.J., **Welch, D.R.** and Tlsty, T.D. Measurement of genomic instability in rat mammary adenocarcinoma cell clones of differing metastatic potential. Loss of Genomic Instability in Neoplasia (1993) 17: 33.

Miele, M.E., Chen, P., Weissman, B.E., Stanbridge, E.J., and **Welch, D.R.** Suppression of metastasis following introduction of chromosome 6 does not correlate with SOD2 expression. The Second International Chromosome 6 Mapping Workshop. Berlin, Germany.

Miele, M.E., Gresham, V.C., Stanbridge, E.J., Weissman, B.E. and **Welch, D.R.** Metastasis, but not tumorigenicity, is suppressed and Nm23 levels are increased by introduction of chromosome 6 into human malignant melanoma cell line C8161. Proceedings of the American Association for Cancer Research (1994) 35: 325. [*winner of Brigid Leventhal Award*]

McGary, C.T. and **Welch, D.R.** Stimulation of bone marrow by highly metastatic 13762NF rat mammary carcinoma cell clones. Proceedings of the American Association for Cancer Research (1994) 35: 326.

McGary, C.T. and **Welch, D.R.** Differential protein expression in tumor-elicited mammary carcinoma metastasis-enhancing neutrophils. Proceedings American Association for Cancer Research (1994) 35: 327.

Coleman, A., Miele, M.E., Robertson, G., Lugo, T.G. and **Welch, D.R.** Experimental metastasis is apparently suppressed in human melanoma cell line MelJuSo by introduction of human chromosome 6. Clinical and Experimental Metastasis (1994) 12: 76.

Freiberg, A.S., Stine, C.J., McGary, C.T., and **Welch, D.R.** Development and characterization of a human neuroblastoma model of liver metastasis. Clinical and Experimental Metastasis (1994) 12: 210.

Welch, D.R., Miele, M.E., Lee, J.-H., Weissman, B.E., Coleman, A., Robertson, G., Jiang, H., Lin, J., Fisher, P.B., and Lugo, T.G. Use of microcell-mediated chromosome transfer to identify human melanoma metastasis-suppressor genes. Clinical and Experimental Metastasis (1994) 12: 8.

Grenko, R.T., **Welch, D.R.** and Van Kooten, D. Immunodetection of neural cell adhesion molecule, p53 and cathepsin D in salivary gland adenoid cystic carcinoma. *American Journal of Clinical Pathology*, submitted.

McGary, C.T. and **Welch, D.R.** Highly metastatic 13762NF rat mammary adenocarcinoma clones secrete IL-3 or GM-CSF like activity. Proceedings of the American Association for Cancer Research (1995) 36: 456.

Miele, M.E. and **Welch, D.R.** Transfection of SOD2 into a highly metastatic human melanoma cell line C8161 does not alter tumorigenicity or metastatic ability. Proceedings of the American Association for Cancer Research (1995) 36: 455

Rieber, M., Rieber, M.S., Miele, M.E. and **Welch, D.R.** BrdU-induced growth arrest in metastatic p53^{mut} human melanoma cells involves increased p21/WAF1/CIP1 and p110^{Rb} with concurrent decline in cyclin A. Proceedings of the American Association for Cancer Research (1995) 36: 3154

Dong, C., You, J., Miele, M.E. and **Welch, D.R.** Tumor cell motility in the metastatic cascade can be regulated

by genes on chromosome 6. Biorheology (1995) 32: 203.

Kiley, S., **Welch, D.R.** and Jaken, S. Protein kinase C (PKC) isozyme and substrate expression changes with progressive metastatic potential in mammary epithelial cell lines. 9th International Conference on Second Messengers and Phosphoproteins (1995).

Welch, D.R., Miele, M.E., Lee, J.-H., Robertson,G., Coleman, A., Weissman, B.E., Fisher, P.B., Lugo, T.G. Evidence for melanoma metastasis-suppressor genes on human chromosomes 1 and 6. 5th International Congress of Anticancer Research (1995).

Miele M.E., De La Rosa A., Steeg P.S., **Welch, D.R.** NME1 (Nm23-H1) levels rise in metastasis-suppressed chromosome 6/human melanoma hybrids, but transfection with NME1 does not completely suppress metastasis. Proceedings of the American Association for Cancer Research (1996) 37: 528.

Lee J-H., Doumen D.J. and **Welch D.R.** Cloning of a novel gene, KiSS-1, which is responsible for metastasis suppression in chromosome 6/human melanoma hybrid cells. Proceedings of the American Association for Cancer Research (1996) 37: 531.

Lee J.-H. and **Welch D.R.** Use of differential display and subtractive hybridization to identify highly expressed genes in metastasis-suppressed chromosome 6/human melanoma hybrids. Proceedings of the American Association for Cancer Research (1996) 37: 530.

Miele ME, Robertson G, Lee J-H, A. Coleman, McGary CT, Fisher PB , Lugo T G and **Welch D R** Metastasis is suppressed in human melanoma cell line MelJuSo following introduction of chromosomes 1 or 6 but tumorigenicity and local invasiveness are unaffected. Proceedings of the American Association for Cancer Research (1996) 37: 527.

Wei, L.L., Yang, X., Phillips, K., Weissman, B.E., **Welch, D.R.** Analysis of KAI-1 mRNA expression in human breast cancer cell lines. Proceedings of the American Association for Cancer Research (1996) 37: 529.

Phillips, K. K., **Welch, D.R.**, Miele, M.E., Lee, J.-H., Wei, L.L., and Weissman, B.E. Suppression of MDA-MB-435 breast carcinoma cell metastasis following the introduction of human chromosome 11. Proceedings of the American Association for Cancer Research (1996) 37: 568.

Kiley, S., **Welch, D.R.** and Jaken, S. W. Protein kinase Cδ: use of a dominant-negative strategy to study its contribution to metastatic progression in mammary epithelial cells. Proceedings of the American Association for Cancer Research (1996) 37: 1059.

Miele, M.E., Goldberg, S.F., Hyatt, D.L., Weissman, B.E., Barbanti-Brodano, G., **Welch, D.R.** Human melanoma metastasis-suppressor locus maps to 6q16.3-q23. Proceedings of the American Association for Cancer Research (1997) 38: 1361.

Lee, J.-H., Goldberg, S.F., Hicks, D.J., **Welch, D.R.** Suppression of human breast carcinoma MDA-MB-435 tumor growth and metastasis by KiSS-1. Proceedings of the American Association for Cancer Research (1997) 38: 545.

Kiley, S., Goodnough, M., Clark, K., **Welch, D.R.**, Jaken, S. Protein kinase C δ promotes mammary tumor progression. Proceedings of the American Association for Cancer Research (1997) 38: 2500.

Jaken, S., Kiley, S., Clark, K., Goodenough, M., **Welch, D.** and Medina, D. Protein kinase C in mammary carcinogenesis. Vermont Cancer Meetings (1997). 1:1.

Welch, D.R. and Lee, J.-H. Isolation and initial characterization of KiSS-1, a human metastasis-suppressor gene, Cold Spring Harbor Symposium on Fundamental Cancer Research (1997) pp. 518.

Jaken, S., Kiley, S.C., Medina, D. **Welch, D.R.** Protein kinase C in mammary carcinogenesis. An Era of Hope – U.S. Army Medical Research and Materiel Command Breast Cancer Research Program (1997) 2: 411.

Lee, J.-H., Hicks, D.J., Goldberg, S.F. **Welch, D.R.** Suppression of human breast carcinoma MDA-MB-435 metastasis by the melanoma metastasis-suppressor gene, KiSS-1. An Era of Hope – U.S. Army Medical Research and Materiel Command Breast Cancer Research Program (1997) 2: 715.

Welch, D.R., Lee, J.-H., Miele, M.E., and Weissman, B.E. Identification of metastasis suppressor genes in human cancer. Molecular Determinants of Cancer Metastasis (1997) pp. 71-73.

Kiley, S., Goodnough, M, Clark, K., **Welch, D.R.**, Jaken, S. Dominant negative protein kinase C-δ inhibits the metastatic progression of mammary tumor cells in vivo. Proceedings of the American Association for Cancer Research (1998) 39: 534

Miele, M.E.; Billelo, J.P.; Robertson, G.P.; Huang, H.-J.S.; Cavenee, W.K.; Hicks, D.J.; **Welch, D.R.** PTEN/MMAC1 does not suppress tumorigenicity or metastasis in C8161 human melanoma cells. Proceedings of the American Association for Cancer Research (1998) 39: 4199

Alessandrini, A. and **Welch, D.R.**, Constitutively active MEK1 induces metastatic potential in NIH-3T3 cells. Cold Spring Harbor Meeting – Cancer Genetics and Tumor Suppressor Genes. (1998) p.23.

Fountain, J.W., Karanjawala, Z., Sridhar, A., Chen, L-L., Walker, G.J., Hayward, N.K., **Welch, D.R.**, Rice, A., Kurera, D., Yebha, Y., Glendening, J.M., Goldberg, E.K. Localization of melanoma tumor suppressor genes on chromosome 11 using a novel method, homozygosity mapping of deletions (HOMOD) analysis. Cold Spring Harbor Meeting – Cancer Genetics and Tumor Suppressor Genes. (1998) p.61.

Alessandrini, A. and **Welch, D.R.**, Constitutively active MEK1 induces metastatic potential in NIH-3T3 cells. Journal of Biomolecular Structure and Dynamics (1998) 16: 149.

Alessandrini, A. and **Welch, D.R.** Transfection with constitutively active Mek1 confers tumorigenic and metastatic potential to NIH-3T3 cells. VII International Congress of the Metastasis Research Society (1998)

Barnum-Huckins, K., Cover, C., Selmin, O., Runyan, R., Robertson, G., Lugo, T.G., **Welch, D.R.**, and Nelson, M.A. Chromosome 1 mediated differential gene expression in malignant melanoma. American Society of Human Genetics (1998).

Kiley, S.C., Clark, K., **Welch, D.R.**, and Jaken, S. Dominant-negative protein kinase C-δ inhibits cytoskeletal-dependent processes in metastatic cells. Lake Placid Symposium (September 24-27, 1998; Lake Placid, NY).

Kiley, S.C., Clark, K., **Welch, D.R.**, and Jaken, S. Dominant-negative protein kinase C-δ inhibits cytoskeletal-dependent processes in metastatic cells. American Society of Biochemistry and Molecular Biology – Protein kinase C and cellular function (October 9-12, 1998; Lake Tahoe, CA).

Goldberg, S.F., Hopper, J.E. and **Welch, D.R.** Protein interactors of KiSS1, a suppressor of metastasis. Graduate Student Research Forum (March 12, 1999, Hershey, PA).

Harms, J.F., Goldberg, S.F., Verderame, M.F., and **Welch, D.R.** C8161 melanoma cells suppressed for metastasis form micrometastases but fail to proliferate. Graduate Student Research Forum (March 12, 1999, Hershey, PA).

Alessandrini, A. and **Welch, D.R.** Transfection with constitutively active Mek1 confers tumorigenic and metastatic potential to NIH-3T3 cells. Proceedings of the American Association for Cancer Research (1999) <u>40</u>: 1312.

Verderame, M.F. and **Welch, D.R.** Genetic complementation of a host-dependent v-*src* mutant allele. Proceedings of the American Association for Cancer Research (1999) <u>40</u>: 2453.

Barnum-Huckins, K.M., Cover, C., Robertson, G., **Welch, D.R.**, Selmin, O., Nelson, M.A. Uniquely expressed cDNAs mediated by chromosome 1 in tumorigenicity suppressed melanoma cells. Proceedings of the American Association for Cancer Research (1999) <u>40</u>: 2122.

Rieber, M., **Welch, D.R.**, and Rieber, M.S. Differential gene expression associated with suppression of metastatic melanoma cell spreading and proliferation on adhesion-restrictive substrates. Proceedings of the American Association for Cancer Research (1999) <u>40</u>: 491.

Seraj, Md. J., Samant, R.S., Verderame, M.F., Hicks, D.J., Sakamaki, T., Hwang, C.K. Weissman, B.E., and **Welch, D.R.** Identification of breast cancer metastasis-suppressor genes from metastasis-suppressed chromosome 11/MDA-MB-435 hybrids. Proceedings of the American Association for Cancer Research (1999) <u>40</u>: 689.

Goldberg, S.F., Hopper, J.E. and **Welch, D.R.** Protein interactors of KiSS-1, a suppressor of metastasis. Proceedings of the American Association for Cancer Research (1999) <u>40</u>: 690. [*Recipient of Brigid Leventhal WICR/AACR Travel Award*]

Welch, D.R. Genetic regulation of cancer metastasis. 8th Annual Meeting of the Japanese Association for Metastasis Research (1999) 8: 36.

Welch, D.R., Harms, J.F., Goldberg, S.F., Meehan, W.J., Seraj, M.J., Leonard, T.O., Samant, R.S., Miele, M.E., Lee, J.H., Hicks, D.J. Identifying and characterizing metastasis-suppressor genes in human cancer. Proceedings of the First BACT International Symposium (1999) 1: 32-38.

Welch, D.R., Seraj, M.J., Samant, R.S., Leonard, T.O., Harms, J.F., Verderame, M.F. BrMS1 – A human breast cancer metastasis-suppressor gene encoded on chromosome 11q13.1-q13.2. *Clinical Cancer Research* 5:66.

Harms, J.F., Goldberg, S.F., Quon, K., **Welch, D.R.** Metastasis-suppressed C8161 melanoma cells arrest in lung but fail to proliferate. Proceedings of the American Association for Cancer Research (2000) <u>41</u>: 1462. [*Recipient of Brigid Leventhal WICR/AACR Travel Award*]

Miele, M.E., Goldberg, S.F., Jewett, M.D. **Welch, D.R.** Characterization of a 40 megabase region on the long arm of human chromosome 6 (Chr6) harboring a putative metastasis-suppressor gene. Proceedings of the American Association for Cancer Research (2000) <u>41</u>: 5547.

Leonard, T.O., Hicks, D.J., Fountain, J.W., **Welch, D.R.** Kai1 suppresses metastasis and tumor growth in the human metastatic melanoma cell line MelJuSo. Proceedings of the American Association for Cancer Research (2000) <u>41</u>: 58.

Samant, R.S., Seraj, M.J., Meehan, W.J., Harms, J.F., Leonard, T.O., Shevde, L.A., Sakamaki, T., Winter, C.R., Verderame, M.F., **Welch, D.R.** BrMS1 — a human breast carcinoma metastasis-suppressor gene. Proceedings of the American Association for Cancer Research (2000) <u>41</u>: 1053

Samant, R.S., Debies, M.T., Seraj, M.J., Verderame, M.F., **Welch, D.R.** Genomic organization and chromosomal localization of the breast metastasis suppressor gene [BRMS1]. Proceedings of the American Association for Cancer Research (2000) <u>41</u>: 1461.

Brown, L.M., **Welch, D.R.**, Rannels, D.E., and Rannels, S.R. B16F10 melanoma cell metastasis is enhanced to the lungs of mice subjected to partial pneumonectomy. Proceedings of the American Association for Cancer Research (2000) <u>41</u>: 4669.

Saunders, M.M., Seraj, M.J., Yellowley, C.E., Hoke, A., **Welch, D.R.**, and Donahue, H.J. Gap junctional intercellular communication is restored in metastasis-suppressed breast carcinoma cells. Proceedings of the Federation of the Societies of Experimental Biology 2000 (in press).

Samant, R.S., Seraj, M.J., Shevde, L.A., Sakamaki, T., Meehan, W.J., Leonard, T.O., Harms, J.F., Winter, C.R.., Debies, M.T., Verderame, M.F., and **Welch, D.R.** BRMS1 – a novel human breast cancer metastasis-suppressor gene. 3rd annual regional cancer center consortium for biological therapy of cancer (2000) 3: 15.

Seraj, M.J., Samant, R.S., Shevde, L.A., Saunders, M.M., Sakamaki, T., Meehan, W.J., Donahue, H.J., Debies, M.T., Budgeon, L., Leonard, T.O., Harms, J.F., Christensen, N.D., Winter, C.R., Verderame, M.F., and **Welch, D.R.** BRMS1- a human breast cancer metastasis suppressor gene encoded on chromosome 11q13.1-q13.2. Era of Hope – Department of Defense Breast Cancer Research Program Meeting (2000) 1: 111-112.

PRESENTATIONS AT SCIENTIFIC MEETINGS:

2000   *Tumor-host interactions in cancer metastasis*, VIIIth International Congress of the Metastasis Research Society, London England (9/25)

BRMS1 - a human breast cancer metastasis-suppressor gene encoded on chromosome 11q13.1-q13.2. Era of Hope DOD Breast Cancer Research Program's Meeting, Atlanta, GA (6/9)

Chromosome 6 blocks growth of human melanoma metastases at the secondary site. AACR Special Conference on Melanoma: Basic Biology and Immunological Approaches, The Woodlands, TX (5/6)

Chromosome 6-melanoma hybrids spread to lung but do not proliferate, Innovations in Biological Therapy of cancer "2000" 3rd Annual regional cancer center consortium for biological therapy of cancer. Presented by S. F. Goldberg (2/25).

Strategies to elucidate the genetics of neoplastic progression and metastasis, Molecular Aspects of Growth Control and Apoptosis, Caracas Venezuela (1/27)

In vivo and in vitro methods to study cancer metastasis, Molecular Aspects of Growth Control and Apoptosis, Caracas Venezuela (1/28)

What defines a clinically useful marker of cancer metastasis? Molecular Aspects of Growth Control and Apoptosis, Caracas Venezuela (1/29)

1999   *Metastasis overview and metastasis genes*, Fall Inland Northwest Cancer Conference – Research & Clinical Practice: Bridging the Gap (11/6)

Putative Metastasis-suppressor genes in human melanoma, AACR Special Conference – Molecular Aspects of Metastasis, Snowmass, CO (9/23)

Genes controlling metastasis in human melanoma and breast carcinoma, Japanese Association for Metastasis Research, Tokyo Japan (5/24)

Genetics of cancer metastasis: prospects for therapeutic intervention , 1st International BACT Symposium/ 10th Annual Symposium on the Biological Approaches to Cancer Treatment, Nagoya Japan (5/22)

Identification of breast cancer metastasis-suppressor genes from metastasis-suppressed chromosome 11/MDA-MB-435 hybrids, Integrative Biosciences Symposium, State College, PA (5/7)

Host-tumor interactions in cancer invasion and metastasis: Genetic Implications. American Association for Cancer Research Annual Meeting (4/14)

Transfection with constitutively active Mek1 confers tumorigenic and metastatic potential to NIH-3T3 cells, American Association for Cancer Research Annual Meeting (4/12)

1998   *Genetic regulation of melanoma and breast tumor metastasis,* VII International Congress of the Metastasis
          Research Society, San Diego, CA (10/8)

1997   *Suppression of human breast carcinoma MDA-MB-435 tumor growth and metastasis by KiSS-1,* An Era of
          Hope (U.S. Army Medical Research and Materiel Command – Breast Cancer Program), Washington, D.C.
          (11/2)

         Identification of metastasis-suppressor genes in human cancer 50th Annual Symposium on Fundamental
          Cancer Research, M.D. Anderson Cancer Center, (10/31)

         Isolation and initial characterization of KiSS-1, a human metastasis-suppressor gene, Cold Spring
          Harbor/Frederick Cancer Research Center Symposium on Cancer Genetics and Tumor suppressor genes
          (6/13)

1996   *Identification of metastasis-controlling genes in human cancer,* W. Alton Jones Symposium - Mammary
          Tumor Biology (8/11)

         Identification and initial characterization of a human melanoma metastasis-suppressor gene, KiSS-1, Gordon
          Research Conference on Cancer (8/5)

1995   *Molecular mechanisms of melanoma progression* Fifth International Congress of Anticancer Research (10/95)

         Tumor cell motility in the metastatic cascade can be regulated by genes on chromosome 6. 2nd International
          Congress on Clinical Hemorheology (7/95)

         Highly metastatic 13762NF rat mammary adenocarcinoma clones secrete IL-3 or GM-CSF like activity.
          American Association for Cancer Research (3/22)

1994   *Microcell-mediated chromosome transfer as a method for identifying metastasis-suppressor genes.* Fifth
          Congress of the International Metastasis Research Society, Washington, D.C. (9/29)

         Features distinguishing cirrhosis from hepatocellular carcinoma by fine needle aspiration. Abendroth, C.S.,
          Grenko, R.T. and Welch, D.R., 42nd Annual meeting of the American Society of Cytology, Chicago, IL
          (11/1)

1993   *Suppression of metastasis following introduction of chromosome 6 does not correlate with SOD2 expression*:
          Second International Chromosome 6 Mapping Workshop. Berlin, Germany

         Genomic instability does not increase as tumor cells progress toward malignancy. 17th Annual UNC
          Lineberger Cancer Center Symposium, Chapel Hill, NC

         Mechanisms of Cancer Spread, Cancer Research in the Future: Jake Gittlen Cancer Research Lecture, Hershey,
          PA

         Enhancement of human melanoma metastasis by TNF-α is inhibited by antisense ICAM-1 oligonucleotides.
          American Association for Cancer Research, Orlando, FL

1992   *Theoretical Considerations for Drug Targeting in the Cancer Patient,* United Nations Conference on the
          Molecular Basis for Diagnosis in Human Disease, Caracas, Venezuela

         Chromosome 6 Contains a Tumor Progression Suppressor Gene for Human Melanoma? United Nations
          Conference on the Molecular Basis of Diagnosis in Human Disease, Caracas, Venezuela

1990   *TGF-β Stimulates Lung Colonization by Rat 13762NF Mammary Adenocarcinoma Clone MTLn3.* American
          Association for Cancer Research, Washington, DC

         *Cytokine Modulation of Intercellular Adhesion Molecule-1 Surface Expression on Human Melanoma Cells:
          Correlation with Adhesion of Peripheral Blood Leukocytes.* American Association for Cancer Research,
          Washington, DC

1988   *Screening Systems for Anti-invasive and Anti-metastatic Drugs.* Bioassay-directed Discovery of Anti-tumor
          and Anti-viral Agents from Natural Sources. NAIAD, National Institutes of Health, Bethesda, Maryland

         The Use of Steroids for Preventing Tumor Angiogenesis. Steroids in Cancer Therapy, Montreal, Quebec

         *Experimental Metastatic Potential of 13762NF Mammary Adenocarcinoma Cells is Modulated by Factors
          Secreted by Tumor Cells.* Ontario Metastasis Research Group, Detroit, Michigan

1987   *13762NF Mammary Adenocarcinoma as a Model of Metastasis.* National Institutes of Health, Bethesda,
          Maryland

         Tumor Heterogeneity. FASEB Summer Research Conference - Cancer Metastasis, Saxton's River, Vermont

         Drug Delivery, Tumor Biology and Drug Targeting. Anticancer Therapy: Therapeutic Index Improvement by
          Toxicity Reduction, London, England

1986   *DNA Hypomethylation Reduces Repair of Sublethal Radiation Damage by 13762NF Mammary
          Adenocarcinoma Cells?* Upjohn Biology Symposium

Strategies for the Treatment and/or Prevention of Tumor Progression. Treatment of Metastasis, Trieste, Italy

Locally Recurring Mammary Tumors Exhibit Sensitivities to Chemotherapy Different from Primary Tumors. Treatment of Metastasis, Trieste, Italy

1985 *Discussion of the Suitability, Availability and Requirements for In Vivo and In Vitro Models of Metastasis: Cellular Instability and Heterogeneity.* Cancer Metastasis: Experimental and Clinical Strategies, Kalamazoo, Michigan

1984 *Simultaneous and Independent Drift of Multiple Phenotypes in Mammary Adenocarcinoma Cell Clones.* Treatment of Metastasis: Problems and Prospects, London, England

1983 *Heterogeneity and Instability of Metastatic Tumor Cell Response to Radiation and Chemotherapy In Vitro.* Tissue Culture Association - Texas Branch, Spring Meeting, Houston, Texas

1982 *Heterogeneous Response of 13762NF Mammary Adenocarcinoma Clones to Gamma Radiation In Vitro.* Tissue Culture Association - Texas Branch, Spring Meeting, Houston, Texas

1981 *Biological and Biochemical Properties of Malignant Sublines and Clones of a Spontaneously Metastasizing Mammary Adenocarcinoma.* 4th Annual San Antonio Breast Cancer Symposium, San Antonio, Texas

INVITED LECTURES:

2000 *Metastasis suppressor genes in human cancer: from discovery to mechanism of action.* University of New Mexico Health Science Center; Steve Schiff Skin Cancer Research Institute (6/3)

Metastasis Suppressor Genes in Human Cancer, Life Sciences Consortium Graduate Program in Nutrition, Penn State University, February 15.

Molecular mechanisms of cancer metastasis, University of Rochester Cancer Center, January 24

Genetics of breast cancer metastasis, Penn State Cancer Center, January 17

1999 *Molecular regulation of metastasis – opportunities for clinical intervention*, Genzyme Inc., December 6

New targets for treating cancer – metastasis and genomic instability, Council for the Advancement of Science Writing Inc., November 8

Genes that control cancer metastasis, Pennsylvania Cancer Registry, September 29

Identifying and characterizing human metastasis-suppressor genes. Novartis Pharma, May 27

Regulation of Metastasis in Human Cancers, University of Tokyo, May 26

So You Chose a Career in Science?, Desert High School, April 30

Metastasis suppression in human cancer, University of Chicago Cancer Center, April 28

Melanoma Metastasis, Wistar Institute, March 23

How do I know what specialty to do? Christian Medical Society, Penn State University, March 18

Update on the genetics of cancer metastasis. Hematology/Oncology Grand Rounds, Penn State Geisinger Health System, March 17

Melanoma Genetics, Penn State Geisinger Cancer Center Interdisciplinary Conference, March 15

Tumor angiogenesis and metastasis: Therapeutic options. Pennsylvania Society of Oncology Nurses, Hershey, PA, February 24

The molecular basis of human melanoma metastasis (Co-lecturer, S.F. Goldberg), Penn State Geisinger Health System, January 28

1998 *Cancer Metastasis – Opportunities for Bioengineering Research*, Penn State University, December 8

Molecular mechanisms controlling cancer metastasis. University of Texas-Houston Medical School, November 18

Cancer Research Update, WLBR Radio-Don Bowman Show, August 17

Molecular regulation of melanoma and breast carcinoma metastasis, Wake Forest University Cancer Center, July 28

Molecular mechanisms controlling melanoma and breast carcinoma metastasis. University of Chicago Cancer Center, May 15

Are there careers available for me in cancer research? Penn State University Continuing Education, Career Day, May 5

How do I know the right thing to do? Christian Medical Society, Penn State University, May 1

Molecular basis of cancer metastasis, Massachusetts General Hospital, March 18

KiSS-1, a novel human metastasis-suppressor gene, Genzyme, Inc., March 17

1997 *Identification and characterization of human metastasis-controlling genes*, American Health Foundation,

December 5

The molecular basis of cancer metastasis - Spelman College, November 25

KiSS-1, a human metastasis-suppressor gene, University of Texas System Cancer Center - Science Park Research Division, October 30

Breast cancer research – recent findings, Millersville University, October 14

WLBR Radio-Don Bowman Show, *Conquering Cancer with a Quarter-Research Update*, August 19

Identification and possible mechanisms of action of KiSS-1, a novel human metastasis-suppressor gene, Pathology Grand Rounds, University of Rochester, May 30

KiSS-1, a novel human metastasis-suppressor gene, Surgical Grand Rounds, St. Luke's Hospital, Bethlehem, PA, May 28

Involvement of protein kinase C isoforms in human cancer metastasis control, Endocrinology Research Conference, Penn State University College of Medicine, May 22

1996   *Identification and initial characterization of a novel human melanoma metastasis-suppressor gene, KiSS-1*, Roswell Park Cancer Institute, December 11

Identification and initial characterization of KiSS-1, a novel human melanoma metastasis-suppressor gene M.D. Anderson Cancer Center, November 19

Metastasis-suppressor genes: an opportunity for therapeutic intervention?, American Cancer Society-Commonwealth Division, October 17

Toward a molecular understanding of melanoma metastasis, Penn State University Bioengineering Program, November 13

Virology In-service, Penn State College of Medicine, *What is a tumor cell?*, August 22

WLBR Radio-Don Bowman Show, *Conquering Cancer with a Quarter-Research Update*, August 20

Penn State University Cancer Center, *Are antisense oligonucleotides potentially useful for melanoma therapy?*, June 17

Penn State University Cancer Center, *Genetics of breast cancer metastasis*, April 15

National Institutes of Environmental Health Sciences, *KiSS-1, a novel human melanoma metastasis-suppressor gene*, March 21

University of North Carolina-Chapel Hill, Lineberger Cancer Symposium, *KiSS-1, a novel human melanoma metastasis-suppressor gene*, March 20

Lower Dauphin Advanced Placement Biology Classes, *Cancer Research*, March 19

Cell and Molecular Biology Graduate Program, Penn State University, *Novel metastasis-suppressor genes in human melanoma and breast carcinoma*, March 6

1995   Sandoz Pharmaceutical Research, *Novel metastasis-suppressor genes in human cancer: Therapeutic potential*, November 30

Berlex Pharmaceuticals, *Novel metastasis-suppressor genes in human cancer: Therapeutic potential*, November 13

Department of Microbiology & Immunology, M.S. Hershey Medical Center, *Evidence for novel metastasis-suppressor genes in human cancer*, September 28

Department of Dermatology, M.S. Hershey Medical Center, *Genetics of malignant melanoma metastasis.* August 3

WLBR Radio-Don Bowman Show, *Conquering Cancer with a Quarter-Research Update*, August 22

Mission 2000: Breast Cancer Detection and Treatment Goals. District Council-American Cancer Society Pennsylvania Division, May 10

Lombardi Cancer Center, Georgetown University, *Toward a genetic understanding of melanoma metastasis*, February 17

Continuing Education Conference, Evangelical Free Church of America, *Team-building/Small groups/Developing Accountability Relationships* February 4

Institute for Christian Education, *Developing Your Leadership Style*, January 18-February 22

1994   Reach for Recovery Program, American Cancer Society, *Breast Cancer Update*, November 10

Christian Medical Society, Penn State University, *Decision making*, November 9

W. Alton Jones Cell Center, *Molecular mechanisms controlling melanoma metastasis*, November 3

University of Alabama-Birmingham, *Molecular Basis of Cancer Metastasis*, October 27

Department of Biochemistry, Penn State University, *Molecular Mechanisms of Metastasis*, October 3

Division of Endocrinology, Department of Medicine, Penn State University, *Molecular mechanisms of Melanoma Metastasis, An Update*, September 22

Amgen, Inc. *Regulation of Malignant Melanoma Cancer Metastasis*, August 26

WLBR Radio-Don Bowman Show, *Conquering Cancer with a Quarter-Research Update*, August 23

Perry PA, American Cancer Society Annual Meeting, May 25, *The Role of Volunteerism and Contributors in Cancer Research*

Chester PA, American Cancer Society Board of Directors Meeting, May 25, *The Role of Volunteerism and Contributors in Cancer Research*

Genetic & Epigenetic Factors Regulating Cancer Metastasis, Louisiana State University Medical Center, Shreveport, April 19

Malignant Melanoma Metastasis-associated Genes, ISIS Pharmaceuticals, April 7

1993   *Molecular Biology of Skin Cancers*, University of Innsbruck, September 17

Genetic Basis of Melanoma Metastasis, Penn State University Molecular Biology Club, October 13

Biophysical properties of metastatic cancer cells, Penn State University Bioengineering Program, September 23

WLBR Radio-Don Bowman Show, *Conquering Cancer with a Quarter-Research Update*, August 17

Schering-Plough Pharmaceutical Research, *Molecular Basis of Cancer Metastasis*, August 4

DuPont-Merck Research, *Cancer Metastasis as a Therapeutic Target?*, May 3

Lebanon Valley College, *Cancer: To be or not to be*, April 19

American Cancer Society Pennsylvania Division, April 1993, *Panel Discussion on ACS Research*

Johns Hopkins University School of Medicine, May 11, *Molecular Basis of Cancer Metastasis*

Lebanon PA American Cancer Society Board of Directors Meeting, May 25, *The Role of Volunteerism and Contributors in Cancer Research*

Lebanon Valley College, April 19, *Cancer: To be or not to be?*

University of Texas-Health Science Center at Houston, MD/PhD Visiting Lecturer, April 1, *Molecular Basis of Tumor Metastasis*

University of Texas-M.D. Anderson Cancer Center, April 2, *Genetic Regulation of Melanoma Metastasis*

Robert Wood Johnson Pharmaceutical Research Institute, February 11, *Multigene control of cancer metastasis.*

1992   Medical College of Virginia, November 4, *Regulation of Cancer Metastasis*, Grand Rounds

Medical College of Pennsylvania, June 9, *Regulation of Metastasis*

Michigan Cancer Foundation, May 28, *Genetic Basis of Metastasis*

University of California - Riverside, May 14, *Regulation of Tumor Progression and Metastasis*

Canji, Inc., May 15, *Genetic Regulation of Melanoma Metastasis*

Osler Institute, May 25, *Pathology of Neoplasia*

Osler Institute, April 20, *Pathology of Neoplasia*

Cumberland Unit, American Cancer Society, April 19; *Breast Cancer Research*

Ministerio de Sanidad y Asistencia Social (Caracas, Venezuela), February 5, *The Use of Microcell Hybridization to Identify Human Melanoma Metastasis Suppressor Genes*

Instituto Venezolano de Investigaciones Cientificas, February 3, *Paracrine Factors Regulating Mammary Tumor Metastatic Potential*

1991   Burroughs Wellcome Co., April 25, *The Genetics of Malignancy*

1990   Department of Pathology, Pennsylvania State University School of Medicine, March 26, *Genetic and Epigenetic Regulation of Tumor Progression and Metastasis*

Beth Israel Hospital, Harvard Medical School, January 25, *Genetic and Epigenetic Regulation of Tumor Progression and Metastasis*

1989   University of North Carolina at Chapel Hill, December 8, *Genetic and Epigenetic Control of Tumor Progression and Metastasis*

1988   Purdue University, October 4, *The Potential Role for Noncytotoxic Treatments of Cancer Metastasis*

University of Arizona College of Medicine, August 20, *Strategies for Controlling Metastatic Potential and Tumor Progression*

University of Minnesota, May 12, *Strategies for Developing Anti-invasive and Antimetastatic Drugs*

University of Tennessee College of Dentistry, April 19, *Strategies for Developing Antimetastatic Drugs*

1987   Royal Society of Medicine, April 7, *Biologic Considerations for Drug Targeting in Cancer Patients*

        Arizona Cancer Center, October 28, *Designing Cancer Therapy to Account for Changes in Tumor Biology/Progression*

1986   Upjohn Research Colloquium, October 15, *Characterization of Locally Recurring Mammary Tumors*

        Spelman College, October 22, *Strategies for Designing Cancer Therapy to Minimize the Effects of Tumor Progression on Cancer Therapy*

        Institute for Cancer Research, University of Vienna, April 9, *Strategies for Minimizing the Effects of Tumor Progression on Cancer Therapy*

        University of Zagreb, March 31, *Strategies for Minimizing the Effects of Tumor Progression in Cancer Therapy*

        Institute for Oncology, March 28, *Strategies for Minimizing the Effects of Tumor Progression in Cancer Therapy*

1985   Michigan Cancer Foundation, January 25, *Mammary Tumor Progression: Its Role in Cancer Treatment*

1983   Spring Branch Independent School District Continuing Education Inservice Course in Science, August 15, *The Biology of Heart and Cardiovascular Diseases*

        University of Texas Health Science Center, Graduate School of Biomedical Sciences Student Research Symposium, March 31, *Metastatic Tumor Cell Heterogeneity and Instability*

        Graduate School of Biomedical Sciences High School Teacher's Workshop, March 23, *The Biology of Heart Disease*

1982   University of Southern California Comprehensive Cancer Center, December 29, *Effect of Tumor Heterogeneity and Progression on Cancer Therapy*

What Defines a Useful Marker of Metastasis in Human Cancer?

Danny R. Welch, Carrie W. Rinker-Schaeffer

In this issue of the Journal, O'Connell et al. *(1)* describe the identification of a region on the long arm of chromosome 14 that is apparently involved in the progression of breast cancer toward metastasis. Measuring loss of heterozygosity, the investigators found that the majority of lymph node-negative breast tumors did not amplify a region linked to D14S62 and D14S51, while lymph node-positive breast tumors retained heterozygosity for these same markers. These data could imply the existence of a metastasis-promoting gene. Alternatively, the observed molecular changes may be a marker of metastatic propensity.

Since metastasis is the most lethal attribute of a cancer, it is critical that tumors be diagnosed while still localized to achieve the highest probability of long-term survival and quality of life. In the absence of objective evidence that metastases do not exist, earlier diagnosis would accomplish three things: 1) increase the probability of diagnosis prior to spread, 2) decrease total tumor burden so that less therapy is required, and 3) decrease the likelihood of therapy-resistant tumor cell populations.

Detection of cancer has improved appreciably in recent years. However, there is still a critical need for markers that unambiguously distinguish weakly metastatic from highly metastatic lesions. This concept is underscored by the example of malignant melanoma where there is a direct relationship between primary lesion thickness and the likelihood of metastasis *(2)*. For lesions that are overtly thin or thick, planning treatment is easy. However, for lesions of intermediate thickness, the decision is not straightforward. The subjectivity of the current grading criteria is demonstrated by the greater than 50% discordance in the diagnosis and staging of melanomas, even between preeminent dermatopathologists *(3)*.

Choosing useful markers of metastasis requires a better understanding of the metastatic process (Fig. 1) and of how it is distinct from tumorigenicity. Tumorigenicity and oncogenicity refer to the ability of cells to proliferate continuously in the absence of the persistent stimulation by a triggering agent. Tumor progression is the evolution of already tumorigenic cells toward increasingly autonomous states (i.e., decreased dependence on host-derived growth factors and/or increased resistance to negative regulatory molecules). The distinction between oncogenesis and tumor progression is critical when one is determining whether a gene is important in controlling steps associated with malignancy or is simply involved in tumor formation [reviewed in *(4–6)*]. Some of the distinctions between malignant and metastatic are more subtle. Attributes of malignant cells include, but are not limited to, less differentiated morphology/cytology, vascular density, necrosis, high mitotic index, aneuploidy, and high nuclear:cytoplasmic ratio *(7)*. The utility of these characteristics as markers is limited by some degree of subjectivity. In the end, the only incontrovertible hallmark of malignancy is the ability to invade through basement membrane and/or to metastasize.

What characteristics define a suitable marker of metastasis? In general, markers fall into two categories. The first category predicts metastatic propensity based on expression and/or activity of a molecule with an established role in metastasis. For example, matrix metalloproteinases would be expected to be more highly expressed in invasive and metastatic tumors than in their nonmetastatic counterparts. The second category includes markers for which there is no established mechanistic association with metastasis. This category includes known genes with potentially novel functions, novel genes, and molecular changes that correlate with metastatic ability. The markers reported by O'Connell et al. fall into this category, as do the vast majority of markers utilized today [reviewed in *(8,9)*].

Each of these categories can be further divided on the basis of on whether the marker is increased or decreased. This criterion impacts the clinical utility of the marker. Assay sensitivity for molecules that are more highly expressed in metastatic primary tumors would be greater than for those expressed at lower levels because of tumor heterogeneity. It is well recognized that the majority of cells within a tumor cannot complete the multistep process of metastasis. Indeed, less than 0.1% of cells entering the bloodstream successfully form clinically detectable lesions *(7)*. By inference, it follows that a similarly small percentage of cells within a primary tumor would display a marker of metastasis. Just as it is easier to see a single lighted candle in a dark room than to find the only unlit candle in a room full of lighted candles, it is easier to identify a single cell expressing a new marker against a background of nonexpressing cells than it is to find nonexpressing cells within a mass of cells that express a particular marker. This comparison does not even take into account quantitative differences in expression, which would further complicate the matter. For this reason, identification of metastasis-associated, positive regulators would be preferred by pathologists. Examples of such positive regulators include vascular endothelial growth factor, Ras, Mts1, Mta1, and Tiam1 [reviewed in *(5,10)*].

From experimental and treatment perspectives, however, identification of suppressors of metastasis offers advantages. To metastasize, cells must complete all steps of the metastatic cascade shown in Fig. 1. If a cell fails to complete any of these steps, it is nonmetastatic. Thus, it takes only one gene to block metastasis, whereas it takes the coordinated expression of many genes to allow metastasis *(6,11)*. In experimental systems, it is fairly easy to find associations with metastatic ability; however, it is difficult to prove that a particular gene is essential. For example, if one were to transfect a *bona fide* metastasis-
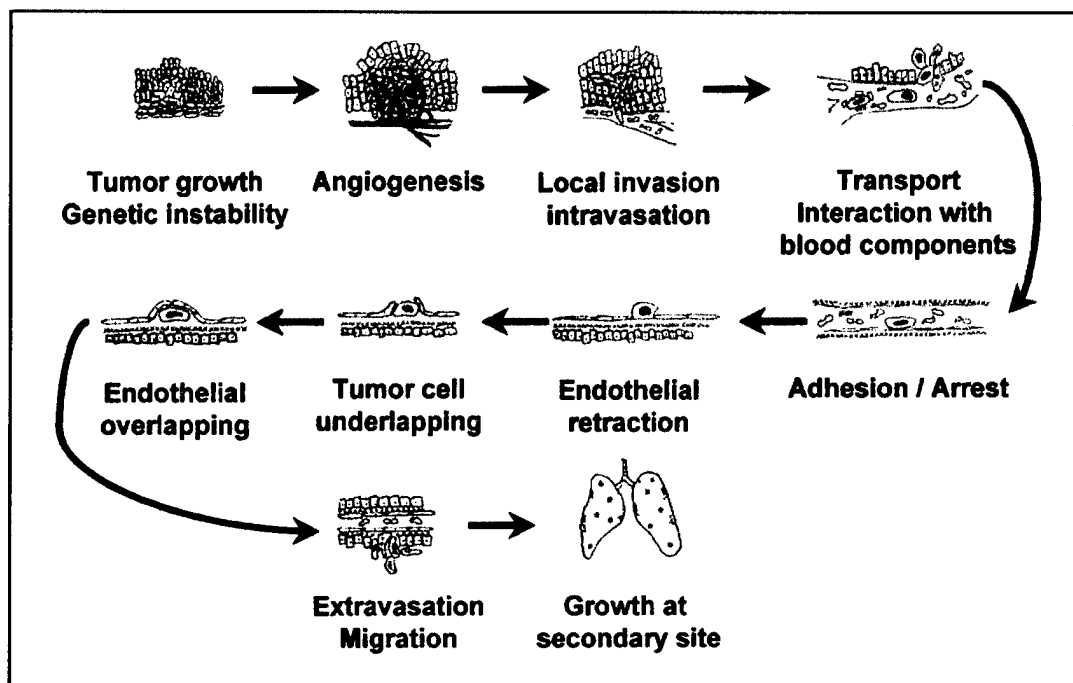
Affiliations of authors: D. R. Welch, Jake Gittlen Cancer Research Institute, The Pennsylvania State University College of Medicine, Hershey; C. W. Rinker-Schaeffer, Section of Urology, Prostate Cancer Program, The University of Chicago, IL.

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See "Note" following "References."

Fig. 1. Pathogenesis of hematogenous metastasis. Metastasis is defined as the formation of secondary tumor foci at a site discontinuous from the primary tumor. Metastases can form following invasion and penetration into adjacent tissues followed by dissemination of cells in the lymphatics, blood vasculature (shown here), coelomic cavities, or epithelial cavities. Metastatic cells arise within a population of neoplastic/tumorigenic cells as a result of genomic instability. This subset of cells has accumulated mutations in addition to those that have already rendered the cells tumorigenic. Metastasis-competent cells have evolved so that they detach and migrate away from the primary tumor. During transport, cells travel individually or as emboli composed of tumor cells (homotypic) or of tumor cells and host cells (heterotypic). At the secondary site, cells or emboli arrest either because of physical limitations (i.e., too large to traverse a lumen) or by binding to specific molecules in particular organs or tissues. Once there, tumor cells then proliferate either in the vasculature or in the surrounding tissue after extravasation. To form macroscopic, clinically detectable metastases (on the order of mm), cells recruit a vascular supply. To form clinically important metastases, cells must complete every step of this complex cascade and proliferate at the secondary site [Fig. adapted from (21)].

Tumor growth Genetic instability → **Angiogenesis** → **Local invasion intravasation** → **Transport Interaction with blood components**

Endothelial overlapping ← **Tumor cell underlapping** ← **Endothelial retraction** ← **Adhesion / Arrest**

Extravasation Migration → **Growth at secondary site**

promoting gene that promoted invasion into a cell that already contained a defect in another gene—say, one required for angiogenesis—that transfected cell would still be nonmetastatic. In contrast, introduction of a gene that disrupts any step in the metastatic cascade would render metastatic cells nonmetastatic. Thus, from a treatment perspective, identification of metastasis-suppressing genes/products would offer the greatest advantage. To date, five such human metastasis suppressor genes have been reported: NME1 *(12,13)*, KiSS1 *(14,15)*, KAI1 *(16,17)*, E-cadherin *(18,19)*, and MKK4 *(20)*.

It must be emphasized that markers can be clinically useful even if their biologic function is not well defined. Indeed, we anticipate that, as a result of efforts to sequence the human genome, there will be numerous molecular markers identified in regions that have not previously been linked to gross chromosomal changes. This concept is well illustrated by the report by O'Connell et al. *(1)*. The immediate, critical concern for breast cancer, as well as for other cancers, is to improve the ability of the pathologist to segregate metastatic from nonmetastatic lesions unambiguously. If it is certain that the patient does not have metastases (even occult ones), treatment beyond surgical removal is not necessary. However, if metastases are present, then more aggressive treatments must begin. As with most issues, decisions are more difficult when the evidence is not clear (i.e., patients with no evidence of macroscopic metastases but for which microscopic, occult metastases are suspected). How are those patients to be treated? The underlying question relates to the level of assurance on which the suspicions are founded. In other words, how confident is the physician that the marker accurately reflects tumor stage? The goal is to avoid unnecessary, expensive treatment, which often comes with undesirable side effects.

As efforts continue toward the identification and development of molecular markers of metastasis, we should heed the experiences that tell us that the use of subjective markers of metastasis leads to ambiguity. In addition, the complexity of the metastatic process suggests that multiple markers may be needed.

REFERENCES

(1) O'Connell P, Fischbach K, Hilsenbeck S, Mohsin SK, Fuqua SA, Clark GM, et al. Loss of heterozygosity at D14S62 and metastatic potential of breast cancer. J Natl Cancer Inst 1999;91:1391-7.

(2) Balch CM, Houghton AN, Milton GW, Sober AJ, Song SJ. Cutaneous melanoma. Philadelphia (PA): Lippincott, Williams and Wilkins; 1991.

(3) Farmer ER, Gonin R, Hanna MP. Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi between expert pathologists. Hum Pathol 1996;27:528-31.

(4) Welch DR, Goldberg SF. Molecular mechanisms controlling human melanoma progression and metastasis. Pathobiology 1997;65:311-30.

(5) Welch DR, Wei LL. Genetic and epigenetic regulation of human breast cancer progression and metastasis. Endocrine-related Cancer 1998;5: 155-97.

(6) Fidler IJ, Radinsky R. Search for genes that suppress cancer metastasis [editorial]. J Natl Cancer Inst 1996;88:1700-3.

(7) Fidler IJ. The biology of cancer metastasis or, "you cannot fix it if you do not know how it works." Bioessays 1991;13:551-4.

(8) Bast RC, Desch CE, Hayes DF, Kemeny NE, Ravdin P, Somerfield MR, et al. 1997 update of recommendations for the use of tumor markers in breast and colorectal cancer. J Clin Oncol 1998;16:793-5.

(9) Brandt-Rauf PW, Pincus MR. Molecular markers of carcinogenesis. Pharmacol Ther 1998;77:135-48.

(10) Dear TN, Kefford RF. Molecular oncogenetics of metastasis. Mol Aspects Med 1990;11:243-324.

(11) Fidler IJ, Radinsky R. Genetic control of cancer metastasis [editorial]. J Natl Cancer Inst 1990;82:166-8.

(12) Freije JM, MacDonald NJ, Steeg PS. Differential gene expression in tumor metastasis: Nm23. Curr Top Microbiol Immunol 1996;213(Part 2): 215–32.

(13) Weinstat-Saslow DL, Steeg PS. Angiogenesis and colonization in the tumor metastatic process: basic and applied advances. FASEB J 1994;8: 401–7.

(14) Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, et al. KiSS-1, a novel human malignant melanoma metastasis-suppressor gene [published erratum appears in J Natl Cancer Inst 1997;89:1549]. J Natl Cancer Inst 1996;88:1731–7.

(15) Lee JH, Welch DR. Identification of highly expressed genes in metastasis-suppressed chromosome 6/human malignant melanoma hybrid cells using subtractive hybridization and differential display. Intl J Cancer 1997;71: 1035–44.

(16) Dong JT, Lamb PW, Rinker-Schaeffer CW, Vukanovic J, Ichikawa T, Isaacs JT, et al. KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2. Science 1995;268:884–6.

(17) Phillips KK, White AE, Hicks DJ, Welch DR, Barrett JC, Wei LL, et al. Correlation between reduction of metastasis in the MDA-MB-435 model system and increased expression of the Kai-1 protein. Mol Carcinog 1998; 21:111–20.

(18) Perl AK, Wilgenbus P, Dahl U, Semb H, Christofori G. A causal role for E-cadherin in the transition from adenoma to carcinoma. Nature 1998;392: 190–3.

(19) Christofori G, Semb H. The role of the cell-adhesion molecule E-cadherin as a tumour-suppressor gene. Trends Biochem Sci 1999;24:73–6.

(20) Yoshida BA, Dubauskas Z, Chekmareva MA, Zaucha MM, Christiano TR, Christiano AP, et al. Identification and characterization of candidate prostate cancer metastasis-suppressor genes encoded on human chromosome 17 [abstract]. Proc Am Assoc Cancer Res 1999;40:701.

(21) Welch DR. Tumor progression: analysis of the instability of the metastatic phenotype, response to radiation and chemotherapy. Houston (TX): The University of Texas; 1984.

NOTE

Advances in Brief

Transfection of Constitutively Active Mitogen-activated Protein/Extracellular Signal-regulated Kinase Kinase Confers Tumorigenic and Metastatic Potentials to NIH3T3 Cells[1]

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Abstract

Cellular growth and differentiation are controlled by multiple extracellular signals, many of which activate extracellular signal-regulated kinase (ERK)/mitogen-activated protein (MAP) kinases. Components of the MAP kinase pathways also cause oncogenic transformation in their constitutively active forms. Moreover, expression of activated *ras* can confer metastatic potential upon some cells. Activation of MAP kinases requires phosphorylation of both Thr and Tyr in the catalytic domain by a family of dual-specificity kinases, called MEKs (MAP kinase/ERK kinase). MEK1 is activated by phosphorylation at Ser^{218} and Ser^{222} by Raf. Mutation of these two sites to acidic residues, specifically $[Asp^{218}]$, $[Asp^{218}, Asp^{222}]$, and $[Glu^{218}, Glu^{222}]$, results in constitutively active MEK1. Using these mutant variants of MEK1, we showed previously that transfection of NIH/3T3 or Swiss 3T3 cells causes morphological transformation and increases growth on soft agar, independent of ERK activity. The transformed cell lines show increased expression of matrix metalloproteinases 2 and 9 and cathepsin L, proteinases that have been implicated in the metastatic process. We tested NIH3T3 cells transfected with the $[Asp^{218}]$ or $[Asp^{218}, Asp^{222}]$ for metastatic potential after i.v. injection into athymic mice. Parental 3T3 cells formed no tumors grossly or histologically. However, all MEK1 mutant transformants formed macroscopic metastases. Thus, like activated Ras, MEK1 can confer both tumorigenic and metastatic potential upon NIH3T3 cells. These results refine the mechanism through which *ras* could confer tumorigenic and metastatic potential (*i.e.*, the critical determinants of tumorigenic and metastatic potential are downstream of MEK1).

Introduction

Components of the MAP[3] kinase signaling pathways (*e.g.*, gip2, Ras, and Raf) cause oncogenic transformation in their constitutively active forms (1). Moreover, expression of activated *ras* can confer metastatic potential upon some cells (reviewed in Ref. 2). The purposes of this study were: (*a*) to begin to ascertain what the downstream effectors of ras transformation and ras-induced metastatic

potential are; and (*b*) to use stable Mek1 mutant variants to address whether ERK1/2 activity is essential for these phenotypes.

Expression of constitutively active MEK1 in NIH3T3 fibroblasts results in cellular transformation (3). Activation of MEK1 is accomplished by phosphorylation of serines at positions 218 (S218) and 222 (S222; Ref. 3). To create constitutively active MEK1, S218 and S222 were mutated to aspartic acid, mimicking the phosphorylated/active state. The MEK1-activated mutants were designated DS and DD, where DS is Asp218/Ser222 and DD is Asp218/Asp222. DS and DD clonal cell lines produced colonies when grown in soft agar, an *in vitro* indicator of transformation. However, anchorage-independent growth did not correlate with ERK1/2 activity. The DS (DS2 and DS4) lines exhibited constitutively active ERK1/2, yet yielded fewer colonies compared with DD lines (DD1 and DD3), which had basal ERK1/2 activity. These data suggested that maintenance of transformation was independent of ERK1/2 activity.

Recently, Webb *et al.* (4), using various ras mutants, showed that although tumorigenicity was independent of ERK1/2 activity, metastasis required its activation. Therefore, we wanted to determine whether clonal cell lines that we established previously and that exhibited constitutive or basal levels of ERK1/2 activity could also confer tumorigenicity and/or metastatic potential. Our data show that tumorigenic and metastatic potentials are dependent upon MEK1 activation but appear to be independent of ERK1/2 activity.

Materials and Methods

Analysis of Metastatic Potential of MEK1-transformed Clonal Lines. Single-cell suspensions of DS and DD were made in ice-cold HBSS and were injected into the lateral tail veins of female athymic mice, 3–4 weeks of age, in a total volume of 0.2 ml/mouse. Mice were killed by methoxyflurane (Metofane; Pitman-Moore, Washington Crossing, NJ), followed by cervical dislocation. Complete necropsies were performed, and metastases were quantified as described (5). Lung metastases were counted after fixing whole lungs in a mixture of neutral buffered formalin and Bouin's fixative (5:1). Random 4–6-μm H&E-stained sections were examined. All animal studies were performed according to the guidelines of the NIH, and protocols were approved by the Institutional Animal Care and Use Committee.

Western Blot. Lungs were isolated 27–41 days after i.v. inoculation from NIH3T3-, DS- and DD-injected athymic mice. The tissue was analyzed by Western blotting as described (3) with some modifications. Briefly, lungs were lysed by Dounce homogenization in potassium phosphate buffer [10 mM KPO_4 (pH 7.05), 1 mM EDTA, 5 mM EGTA, 10 mM $MgCl_2$, 50 mM β-glycerophosphate, 1 mM sodium vanadate, 1 mM DTT, 0.5% NP40, 0.1% Brij-3, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml pepstatin A]. Lung metastases were dissected from adjacent normal tissues before further processing. Lysates were centrifuged at 16,000 $\times$ g for 10 min. Supernatants (40 μg) were boiled in 1$\times$ sample buffer [500 mM Tris-HCl (pH 6.8), 10% SDS, 20% glycerol, 0.05% bromphenol blue, and 1% 2-mercapto-
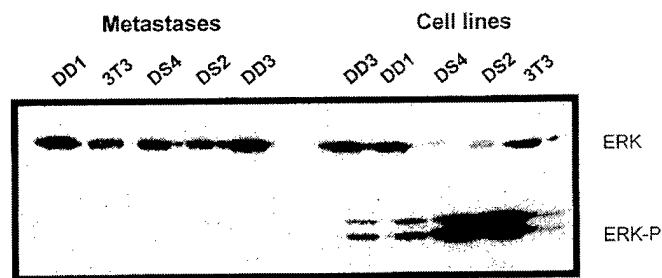
Fig. 1. Differential activation of ERK1/2 by constitutively active MEK1 mutants. Western blot analysis of lysates (40 μg) from MEK1-DS, MEK1-DD, and NIH3T3 cells and lung metastases was performed with phosphospecific-ERK1/2 antibodies (ERK-P). The blot was stripped and reprobed with antibody to total ERK1/2 protein (ERK) to assess equal loading of lysates.

ethanol] for 5 min and electrophoresed on a 10% SDS polyacrylamide gel. Proteins were then transferred to polyvinylidene difluoride Immobilon membrane (Millipore, MA) and probed with phosphospecific ERK1/2 antibody (New England Biolabs, Beverly, MA) at a dilution of 1:1000 at 4°C overnight in PBS containing 0.1% Tween 20 and 3% BSA. Membranes were then incubated with horseradish peroxidase-conjugated antirabbit antibody (Amersham) at a dilution of 1:2000 at room temperature for 20 min, and the signal was detected using electrochemiluminescence (ECL; Amersham), followed by exposure to X-OMAT AR film (Eastman Kodak, Rochester, NY). Blots were stripped and reblotted with anti-ERK1/2 antibody (C-14; Santa Cruz Biotechnology, Biotechnology, CA) to determine equal loading of samples. Stripping was accomplished by submerging the membrane in 100 mM 2-mercaptoethanol, 20% SDS, and 62.5 mM Tris-HCl (pH 6.7) for 30 min at 55°C, followed by washing two times in PBS/0.1% Tween 20 for 10 min.

Growth in Soft Agar and on Bacterial Petri Plates. Cells (1×10^5) were plated onto 60-mm bacterial Petri plates (Fisher Scientific, Pittsburgh, PA) or tissue culture plates (Corning, Oneata, NY) and examined daily for growth and proliferation by counting total cell number using a hemacytometer. The methods used for assessing growth on bacterial plates were identical to those of Rieber et al. (6). Growth in soft agar was done as described (7, 8) using 0.25% agar.

Gelatin Enzymography. Enzymography was done by seeding cells (1.5×10^6/well) in a 12-well plate, followed by incubation at 37°C for 24–48 h. The complete medium was then removed and replaced with serum-free medium, and the cells were incubated at 37°C for 24 h. The next day, the supernatant was removed and spun at 1500 rpm for 10 min. Samples were solubilized in electrophoresis sample buffer containing SDS, absent of β-mercaptoethanol. Samples, normalized to volume and cell number, were loaded onto a 7.5% SDS-PAGE gel containing 1 mg/ml gelatin. After the gel was run, it was transferred to Triton X-100 and incubated for 1 h at room temperature. It was then incubated in reaction buffer for 24 h at 37°C. The gel was stained with Coomassie Blue and destained, and the MMP2 and MMP9 bands were visualized.

Results

MEK1 Mutant Clones Grow on Adhesion-restricted Substrates. DS and DD clones were shown to exhibit differential ERK1/2 activation (Fig. 1). ERK activation levels are consistent with those presented (3). Also, we had shown previously that DS and DD clones produced transformed foci on tissue culture plastic and also produced multicellular colonies in soft agar. Both properties have been correlated with transformation. Recently, Rieber et al. (6) developed an assay in which melanoma cells are seeded onto bacterial
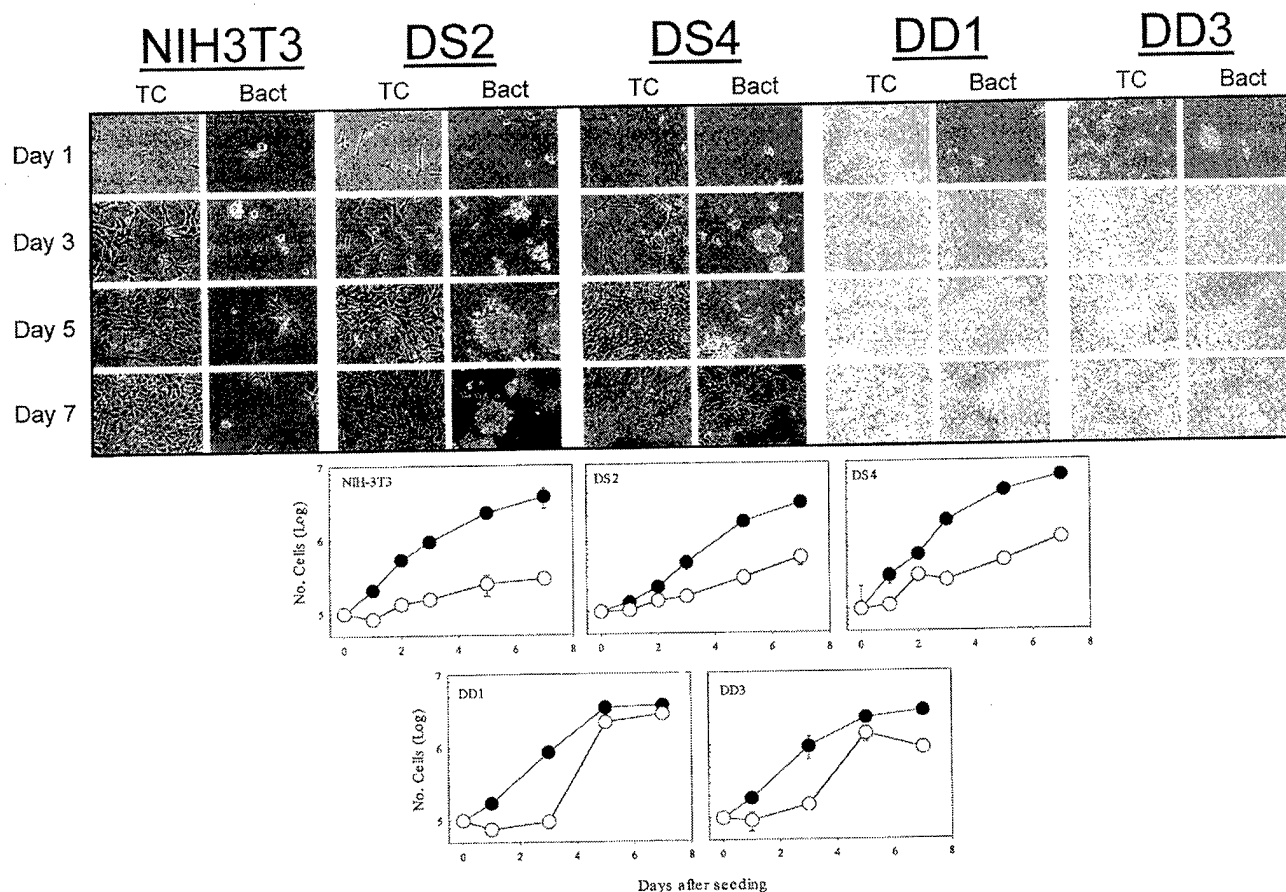

Fig. 2. Growth rates of MEK1 clonal lines on bacterial versus tissue culture plates. A, cells were grown on bacterial (○) and tissue culture (●) dishes and photographed after plating using a Nikon Diaphot microscope at ×40. B, cells were counted at the days indicated. Numbers represent the values from three wells; bars, SE.

Table 1 *MEK1 mutants are metastatic after i.v. inoculation into athymic mice*

Results are shown from two independent experiments. Single-cell suspensions of cells in ice-cold HBSS were injected into the lateral tail vein of 3–4-week-old female athymic mice in a total volume of 0.2 ml/mouse. Upon killing by anesthesia with Metofane followed by cervical dislocation, complete necropsies were done. Metastases were found only in the lungs. To facilitate quantification, lungs were placed into a solution of neutral-buffered formalin and Bouin's fixative (1:5), as described previously (5). Experiment 1 was terminated 41 days after injection. For experiment 2, mice were killed 27 days after injection.

Cell line	No. of cells injected	Incidence of lung metastases	No. of lung metastases/mouse Median (range)	
Experiment 1				
NIH-3T3	5.4×10^5	0/8	0 (−)	0, 0, 0, 0, 0, 0, 0, 0
DD1	5.4×10^5	6.6	>250 (73, >250)	73, 147, >250, >250, >250, >250
DS2	5.4×10^5	4/4	>250 (−)	>250, >250, >250, >250
Experiment 2				
NIH-3T3	3×10^5	0/8	0 (−)	0, 0, 0, 0, 0, 0, 0, 0
DD1	3×10^5	7/8	3.5 (0, 13)	0, 2, 2, 3, 4, 6, 11, 13
DD3	3×10^5	8/8	>250 (−)	>250, >250, >250, >250, >250, >250, >250, >250
DS2	3×10^5	7/7	28 (3, 48)	3, 8, 23, 28, 36, 41, 48
DS4	3×10^6	7/7	47 (17, 136)	17, 23, 30, 47, 49, 71, 136

Petri dishes. Whereas normal melanocytes undergo anoikis under these conditions, tumorigenic cells form spheroid-like masses of non- or poorly proliferating cells. In contrast, metastatic melanoma cell lines adhere to the dishes and proliferate (6). We adapted this assay for use with the MEK1 clonal variants.

NIH3T3, DS, and DD cells survived after seeding onto the bacterial plates. All of the cell lines tended to form spheroid-like structures on the bacterial Petri dishes but were, as expected, spread and exhibited characteristic fibroblast-like morphology on tissue culture plastics (data not shown). NIH3T3 cells, which are immortal but not tumor-igenic, divided once on the bacterial substrate. DS2 and DS4 cells underwent between 2 and 3 cell divisions within 1 week after seeding, whereas DD1 and DD3 cells grew at rates similar to those observed on tissue culture plastic (Fig. 2). Both DD1 and DD3 cells experienced a lag before rapid, exponential growth. We have yet to determine the reasons for this lag, although it is possible that the cells are providing a matrix upon which proliferation can be facilitated.

MEK1 Mutant Clones Are Tumorigenic. On the basis of the *in vitro* transformation phenotypes, DS and DD variants were analyzed for their ability to form tumors after s.c. injection into female athymic mice. Tumors grew rapidly and progressively, detectable within 7–14 days after inoculation with 1 × 10⁶ cells. Parental NIH3T3 cells failed to produce tumors after injection into mice.

DS variant clones exhibited constitutively active ERK1/2 activity whereas the DD clones have basal levels, yet both were equally tumorigenic. This result implies that tumorigenicity is independent of ERK activity. This conclusion is also suggested by Webb *et al.* (4), who used ras transformants.

DD and DS Variants Are Metastatic. Each of the clonal cell lines expressing constitutively active MEK1 formed metastases after i.v. injection into the tail veins of athymic mice. Table 1 shows data from two independent experiments in which the MEK1 clonal lines aggres-sively colonized mouse lungs. These results corroborated and ex-tended previous studies that showed that activated *ras* could confer tumorigenic and metastatic potentials upon NIH3T3 cells (2). Indeed, it showed that at least some portion of these phenotypes were medi-ated through the MAP kinase pathway downstream of MEK1.

On average, DD metastases were slightly but not significantly smaller than DS lesions. In experiment 1, i.v. injection of 5.4 × 10⁵ DD or DS cells produced >250 lung metastases/mouse. In the second experiment, the number of cells injected per mouse was reduced to 3 × 10⁵, and the length of the experiment was shortened (27 days instead of 41 days) to discriminate potential differences more easily. DS2 and DS4 clones that exhibited constitutively active endogenous ERK1/2 (Fig. 1) yielded an average of 28 and 47 lung metastases, respectively (Table 1). DD1 and DD3, which exhibit only basal ERK1/2 activity when compared with NIH3T3 cells (Fig. 1), pro-

duced an average of 5 and >250 lung metastases/mouse, respectively. Other organs did not support metastatic growth, as determined by gross observation and random histological sections. Parental NIH3T3 cells did not develop metastatic nodules in any other tissues. These data suggest that the metastatic potential to lung of the MEK1 clonal lines is independent of initial ERK activity. Metastases resulting from DS and DD clones exhibited characteristics consistent with highly invasive and poorly differentiated fibrosarcoma (Fig. 3A).

Development and/or Maintenance of DS and DD Metastases Does Not Require ERK Activity. Phospho-ERK1/2 activity was measured in the lung metastases isolated from mice in experiment 2. For the DD and DS variants, metastases were dissected from adjacent normal tissue to minimize the impact of activities within the stroma when interpreting the results. Dissection was particularly easy for the DD3 metastases, some of which were >3 mm in diameter. The left set of lanes in Fig. 1 shows that lung metastases isolated from DS2- and DS4-injected mice still exhibited both ERK1 and ERK2 phosphoryl-ation. In contrast, DD1-injected mice exhibited phosphorylation of ERK1, but DD3-injected mice, which yielded the highest number of lung metastases (*e.g.*, >250), showed no detectable ERK1 phospho-rylation, similar to that observed in the lungs of NIH3T3-injected mice. The data suggest that lung colonization is independent of ERK activity. This finding does not eliminate the possibility that ERK is transiently activated during transport in the circulation and/or early colonization.

DS and DD Clones Show Increased MMP2 and MMP9 Activity. Using gelatin enzymography, we demonstrated that the MEK1-trans-formed clonal lines also show an increase in the levels of MMP2 and MMP9 (Fig. 3B), two MMPs that have been implicated in metastasis (9). SDS-PAGE of cell culture supernatants from DS and DD variants also revealed increased levels of a $M_r \sim 39,000$ protein. Isolation and sequencing of this protein revealed that it was cathepsin L.[4] There-fore, it is possible that MEK1 transformation leads to the production of proteinases, such as MMP2, MMP9, and cathepsin L, that contrib-ute to metastatic potential.

Discussion

The molecular basis for cancer metastasis has not been fully elu-cidated. It requires the coordinated expression of multiple genes so that cells migrate from the primary tumor mass, enter a circulatory system, survive transport, arrest at a secondary site, and respond to proliferation signals at the secondary site. Both positive and negative regulatory effectors have been identified (reviewed in Ref. 10).

Previous studies have demonstrated that introduction of oncogenic

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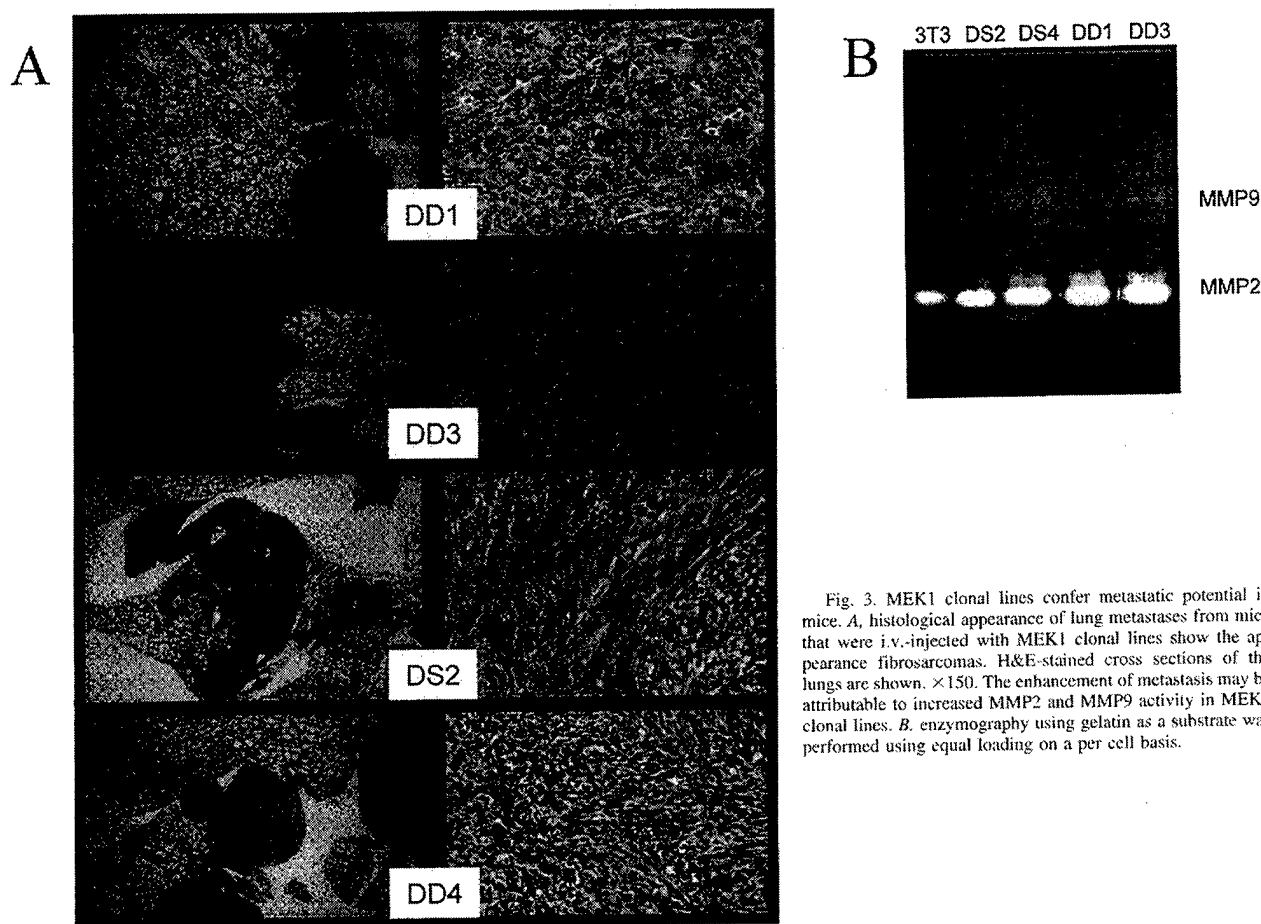[4] Q. Hon, A. Alessandrini, and R. Erikson, unpublished data.

Fig. 3. MEK1 clonal lines confer metastatic potential in mice. A, histological appearance of lung metastases from mice that were i.v.-injected with MEK1 clonal lines show the appearance fibrosarcomas. H&E-stained cross sections of the lungs are shown. ×150. The enhancement of metastasis may be attributable to increased MMP2 and MMP9 activity in MEK1 clonal lines. B, enzymography using gelatin as a substrate was performed using equal loading on a per cell basis.

forms of the *ras* oncogene confer both tumorigenic and metastatic potentials upon NIH3T3 cells. These results implicated events downstream in the regulation of metastasis. The current study was to begin identifying key downstream components contributing to tumorigenicity and metastasis. Toward that end, constitutively active variants of MEK1, a downstream component of the ras/MAPK pathway that has been shown previously to lead to cellular transformation, were used. Like ras, MEK1 transformants of NIH3T3 are not only tumorigenic but are also metastatic. Thus, our results refine the mechanism through which ras can confer these phenotypes and imply that the critical determinants are downstream of MEK1.

Recently, Webb *et al.* (4) used ras transfectants to show that tumorigenicity occurred through both Raf-dependent and -independent pathways. In contrast, metastatic potential in their model correlated with variants that were able to activate ERK1 activity. Seven to 9 weeks after injection into mice, some of the ras transfectants (that were originally nonmetastatic) formed lung colonies. This implies that some selection may have occurred, and the authors suggest that increased expression of the Met receptor tyrosine kinase is responsible, although only modest increases in ERK1/2 activity was observed in these variants. Hepatocyte growth factor, the ligand of the Met receptor, can induce both the MEK1 and phosphatidylinositol 3-kinase pathways, leaving the possibility that the pathway responsible for acquisition of metastasis in these cells is MEK1 and/or phosphatidylinositol 3-kinase dependent.

Although we are in agreement with Webb *et al.* (4) concerning tumorigenicity and how the process may be ERK1/2 independent, we have contrasting results regarding metastatic potential and ERK1/2 activity. This discrepancy may be attributable to the fact that we specifically analyzed MEK1 transformants and not ras

transformants. It is also possible that MEK1-induced transformation may occur through a pathway that is distinct from that of ras transformation and may not necessarily require ERK1/2 activity to maintain transformation.

The current results are consistent with those in which we showed previously that growth in soft agar is independent of ERK activity. DS and DD have varying ERK activity, and we wanted to know whether, in fact, metastasis was dependent upon the level of ERK activity. Western blot data of the lung metastases show that, in fact, metastatic potential is independent of ERK activity, suggesting that the signaling pathway diverges downstream of MEK1, activating as yet undefined components. Although the downstream components have yet to be identified, we do know that these clonal cell lines express and secrete the proteinases MMP2, MMP9, and cathepsin L.

Expression of MMPs has been correlated with metastatic potential as well as ERK, c-Jun NH_2 kinase, and p38 kinase pathways (11), although it was shown previously that stimulation of the MMP9 promoter by ras is independent of MEK1, requiring multiple transcription factor binding sites. Recently, McCawley *et al.* (12) have shown that sustained activation of ERK led to increased MMP-9 activity and cell migration in keratinocytes.

Likewise, increased expression of cathepsin L has been correlated with metastatic potential in ras-transfected cell lines (13). As for the MMPs, higher expression and activity of cathepsin L could lead to higher efficiency penetration of physiological barriers or increased cathepsin L can be involved in tumor cell evasion of immune response by cleavage of the third component of complement (14).

In conclusion, the results presented here refine one pathway involved in the regulation of metastasis by demonstrating that mediators

downstream of MEK1 are involved. Moreover, the results imply that ERK1/2 activity are not essential for the development and/or maintenance of metastatic foci.

References

1. Campbell, S. L., Khosravi-Far, R., Rossman, K. L., Clark, G. J., and Der, C. J. Increasing complexity of Ras signaling. Oncogene, 17: 1395–1413, 1998.
2. Chambers, A. F., and Tuck, A. B. Ras-responsive genes and tumor metastasis. Crit. Rev. Oncog., 4: 95–114, 1993.
3. Alessandrini, A., Greulich, H., Huang, W., and Erikson, R. L. Mek1 phosphorylation site mutants activate Raf-1 in NIH 3T3 cells. J. Biol. Chem., 271: 31612–31618, 1996.
4. Webb, C. P., Van Aelst, L., Wigler, M. H., and Vande, W. G. Signaling pathways in Ras-mediated tumorigenicity and metastasis. Proc. Natl. Acad. Sci. USA, 95: 8773–8778, 1998.
5. Welch, D. R. Technical considerations for studying cancer metastasis in vivo. Clin. Exp. Metastasis, 15: 272–306, 1997.
6. Rieber, M., Welch, D. R., and Strasberg-Rieber, M. Differential gene expression associated with suppression of metastatic melanoma cell spreading and proliferation on adhesion-restrictive substrates. Proc. Am. Assoc. Cancer Res., 40: 491, 1999.
7. Lee, J-H., and Welch, D. R. Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene, KiSS-1. Cancer Res., 57: 2384–2387, 1997.
8. Nicolson, G. L., Lembo, T. M., and Welch, D. R. Growth of rat mammary adenocarcinoma cells in semisolid clonogenic medium not correlated with spontaneous metastatic behavior: heterogeneity in the metastatic, antigenic, enzymatic and drug sensitivity properties of cells from different sized colonies. Cancer Res., 48: 399–404, 1988.
9. Chambers, A. F., and Matrisian, L. M. Changing views of the role of matrix metalloproteinases in metastasis. J. Natl. Cancer Inst., 89: 1260–1270, 1997.
10. Welch, D. R., and Wei, L. L. Genetic and epigenetic regulation of human breast cancer progression and metastasis. Endocr. Relat. Cancer, 5: 155–197, 1998.
11. Ghiso, J. A., Alonso, D. F., Farias, E. F., Gomez, D. E., and Kier Joffe, E. B. Deregulation of the signaling pathways controlling urokinase production. Its relationship with the invasive phenotype. Eur. J. Biochem., 263: 295–304, 1999.
12. McCawley, L. J., Li, S., Wattenberg, E. V., and Hudson, L. G. Sustained activation of the mitogen-activated protein kinase pathway. A mechanism underlying receptor tyrosine kinase specificity for matrix metalloproteinase-9 induction and cell migration. J. Biol. Chem., 274: 4347–4353, 1999.
13. Chambers, A. F., Colella, R., Denhardt, D. T., and Wilson, S. M. Increased expression of cathepsins L and B and decreased activity of their inhibitors in metastatic, ras-transformed NIH 3T3 cells. Mol. Carcinog., 5: 238–245, 1992.
14. Frade, R., Rodrigues-Lima, F., Huang, S. Y., Xie, K. P., Guillaume, N., and Bar-Eli, M. Procathepsin-L, a proteinase that cleaves human C3 (the third component of complement), confers high tumorigenic and metastatic properties to human melanoma cells. Cancer Res., 58: 2733–2736, 1998.

Genetic regulation of human breast carcinoma metastasis

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Running title: Metastasis suppressors in breast cancer

Abstract

This review overviews recent data describing the genetic underpinnings of the suppression of cancer metastasis. Despite the explosion of new information about the genetics of cancer, only six human genes have thus far been shown to functionally suppress metastasis. Not all have been shown functional in breast carcinoma. Several additional genes have been shown to inhibit various steps on the metastatic cascade, but do not necessarily block metastasis when tested using *in vivo* assays. The implications of this are discussed. However, two recently discovered metastasis suppressor genes block proliferation of tumor cells at a secondary site, offering a new target for therapeutic development.

Introduction

Colonization of distant tissues by tumor cells represents the most dangerous attribute of cancer. When breast carcinomas remain confined to breast tissue, cure rates exceed 90%. Yet, as cells spread, long-term survival decreases depending upon the extent of and the sites of colonization. Metastases in visceral organs and brain are the most life-threatening, with 5-year survival rates usually less than 20% [1]. Thus, in order to increase survival, prevention of metastasis and more effective treatment of already established metastases are necessary. Both will be possible only after we have a more thorough understanding of the biological, biochemical and molecular basis of cancer spread. While the focus of this review will be on the genes regulating metastasis, we will briefly review the context under which those genes operate.

Metastasis is defined as the progressive growth of cells at a site discontinuous from the primary tumor. The route of spread is irrelevant in this definition. Cells can disperse via blood vasculature, lymphatics or within body cavities. Metastatic cells are a specialized subset of tumor cells within a primary tumor mass that have acquired the ability to complete the multistep metastatic cascade (reviewed in [2-7]). In brief, these cells migrate, disseminate, extravasate and eventually proliferate at a discontinuous secondary site(s). If a cell fails to complete *any* step in the metastatic cascade, then it is not metastatic.

Failure to metastasize can be due to inherent deficiencies within tumor cells themselves (i.e., genetics) or to defective responses to the host environment (i.e., epigenetics). This concept is not new. Indeed, Stephen Paget advanced the notion over a century ago when he documented the nonrandom distribution of breast carcinoma metastases [8]. Paget's explanation was that the tumor cell or "seed" would grow only when cultivated in an appropriate organ or "soil." His agrarian analogy seems quaint in this era of molecular biology and genomics, but the fundamental principle has withstood the test of time – formation of metastasis depends upon interaction between tumor cells and host cells. Recently, some of the molecules responsible for organ-specific colonization have begun to be elucidated [9-13].

Several questions arise when one contemplates studies of breast cancer genetics, particularly as related to metastasis. How can one expect to define the genetic or biochemical basis of metastasis when it is clear that multiple genes and proteins are involved? And what can be done to compensate for the genomic instability associated with tumor progression? In other words, are we hunting a moving target? Are the same genes responsible for controlling metastasis in different histologic types of breast carcinoma (i.e., infiltrating ductal vs. lobular carcinoma)? How does one identify metastasis-associated genes when environmental context is so important?

Historically, the experimental approach to answering these questions has been reductionist – mimic metastasis component steps (e.g., proliferation, adhesion, invasion, angiogenesis, evasion from immune cell killing, etc.) *in vitro* and study the gene(s) and protein(s) responsible for controlling each step. This approach has led to a tremendous understanding of fine molecular detail for each step, but translation to clinical utility has been limited for the reasons outlined below. Nonetheless, the genetic underpinnings are being elucidated and fundamental biological understanding of the metastatic process is being unraveled.

Oncogenesis and tumor progression are related, but distinct, phenotypes

One area of major confusion regarding metastasis-associated genes has been the failure by some to recognize the important distinction between tumor formation and metastasis. Tumorigenesis and

oncogenesis refer to a cell's ability to proliferate continuously in the absence of persistent stimulation by the triggering carcinogenic agent(s). Tumor progression is the evolution of already tumorigenic cells (populations) towards increasing malignancy. The distinction is crucial when asking whether a gene is important in controlling steps associated with malignancy as compared to whether that gene is involved in tumor formation.

The distinction between *malignant* and *metastatic* is more subtle. Pathologists characterize malignancy based upon several morphologic attributes including: less differentiated cytology, vascularity, necrosis, mitotic index, aneuploidy, and nuclear:cytoplasmic ratio. The incontrovertible hallmarks of malignancy are invasion of cells though a basement membrane and/or metastasis. All other characteristics used to label a tumor (and the cells within it) as malignant have exceptions [14]. For example, morphologically indolent cells may be behaviorally malignant and *vice versa*. Clearly, parameters associated with pathologic examination are invaluable when predicting the probability for local, regional or distant recurrence in a clinical setting [15], but they are limited with regard to cause-effect relationships for genes.

In the context of a multistep, multigenic cascade, it is critical to recognize that the terms invasiveness and adhesion are not equivalent to metastatic propensity. Both invasion and adhesion are necessary, but not sufficient for metastasis. Cells that are efficient at either or both — but which lack the ability to complete *any* other step of the metastatic cascade — are nonmetastatic [16]. Therefore, correlations of genetic expression to a particular step in the metastatic cascade may lead to erroneous conclusions. This can occur in two directions. Inhibition of a step in metastasis, such as invasion, does not necessarily translate to complete inhibition of metastasis *in vivo*. Likewise, at least two recent papers demonstrate that nonmetastatic cells exhibit equal invasiveness (and a variety of other parameters) to their metastatic counterparts [17;18]. The implication is simple – *in vitro* assays are not sufficient surrogates of metastasis to be 100% predictive.

Taken together, these points emphasize the importance for distinguishing each of these phenotypes.

Tumor suppressor genes dominantly inhibit tumor formation when wild-type expression is restored in a neoplastic cell. By definition, then, metastasis would also be suppressed (since the cells are nontumorigenic). *Metastasis-suppressor genes*, on the other hand, block only the ability to form metastases. Restoring expression of a metastasis-suppressor would yield cells which are still tumorigenic, but which are no longer metastatic. From experimental and treatment perspectives, identification of suppressors of metastasis is much simpler than identifying metastasis-promoting genes. This is because it takes only one gene to block metastasis, whereas it takes the coordinated expression of multiple genes to allow metastasis. In experimental systems, it is fairly easy to find associations with metastatic ability; however, it is difficult to prove that a particular gene is essential. For example, if one were to transfect a *bona fide* metastasis-promoting gene (i.e., one that promotes invasion) into a cell that already contains a defect in another gene (for instance, one required for angiogenesis), then the transfected cell would remain nonmetastatic. In contrast, introduction of a gene that disrupts any step in the metastatic cascade would render cells nonmetastatic.

We recently reviewed the literature in breast cancer and found that differential expression of over 150 genes had been correlated with breast cancer development and/or progression [19]. To date, however, only six human metastasis suppressor genes have been demonstrated to have functional activity using *in vivo* metastasis assays: NME1 [20;21], KiSS1 [22;23], KAI1 [24;25], E-cadherin [26;27], BRMS1 [28] and MKK4 [29]. Below we will summarize the key information related to the discovery, activity and mechanisms of action of these metastasis suppressor genes.

Nm23

The first cloned metastasis-suppressor gene, Nm23, was identified in the murine K1735 melanoma using subtractive hybridization because its expression was inversely correlated with lung colonization. The human homolog, Nm23-H1 [also known as NME1], exhibits decreased expression many, but not all, late-stage, metastatic human cancers (reviewed in [20;30]). Decreased expression is the key parameter determining metastatic potential and may occur by a variety of mechanisms not necessarily loss of

heterozygosity (LOH) [30]. Long-term prognostic value has been questioned in some studies [31;32].

Nonetheless, NME1 is a *bona fide* metastasis-suppressor gene in human breast carcinoma since

transfection of metastatic MDA-MB-435 cells suppressed metastasis from an orthotopic site in an

experimental mouse model [33]. Transfection into other cell lines has also resulted in metastasis

suppression [reviewed in [30]], including the human breast carcinoma cell line MDA-MB-435 and the rat

mammary adenocarcinoma MTLn3. *In vitro* assays of control- and Nm23 transfectants have consistently

pointed to decreased motility, invasion and colonization.

The mechanism of action for Nm23 remains unknown. Nm23 is a member of the nucleoside

diphosphate (NDP) kinase family of proteins [34]. NDP kinases are ubiquitous and catalyze the transfer

of γ-phosphates, via a phosphohistidine intermediate, between nucleoside and deoxynucleoside tri- and

diphosphates. However, the NDP kinase activity can be dissociated from its metastasis-suppressor

function [35;36]. Some recent reports suggest that NME1 may control cell cycle progression [37],

histidine-dependent protein phosphorylation [38;39] and transcription [34;40]. The Nm23 story becomes

more complicated because five additional family members have recently been identified and cloned

(Nm23-H2/NME2, Nm23-DR, Nm23-H4, Nm23-H5 and Nm23-H6). Only NME2 has been tested for its

role in metastasis and the results are controversial [41-47].

KiSS1

Metastasis of human melanoma cell lines C8161 and MelJuSo is inhibited following introduction of

an intact human chromosome 6, but tumorigenicity is unaffected [48;49]. KiSS1 was cloned following

subtractive hybridization of comparing mRNA expression in chromosome 6-C8161 and parental C8161

cells. Preliminary data using cell lines indicates that KiSS1 expression is lost as melanoma cells convert

from radial to vertical growth phase (benign to malignant transformation) [22], but more extensive

clinical studies have been slowed due to lack of suitable antibodies.

Since KiSS1 maps to chromosome 1q32 [22;50] and since deletions and rearrangements of the long

arm of chromosome 1 have been associated with breast cancer progression [19], we tested whether KiSS1 would suppress metastasis of the human breast ductal carcinoma cell line MDA-MB-435 which do not express KiSS1. Transfection resulted in suppression of metastasis from the mammary fat pad of athymic mice; whereas, vector-only transfectants were unaffected [51]. Likewise, tumorigenicity was not suppressed.

The mechanism of action for KiSS1 has not yet been determined although its ability to suppress metastasis has been demonstrated in six independently-derived human cancer cell lines of melanoma and breast origin [22;23;51]. Based upon the cDNA sequence, the predicted KiSS1 protein would be a hydrophilic, 164 amino acid protein with molecular mass of 15.4 kDa. The sequence is novel, having no strong homology to any known human cDNA sequences. A recent report suggests that KiSS1 may differentially regulate MMPs. Yan and Boyd recently showed that KiSS1-transfected HT1080 cells showed specific downregulation of MMP9 transcription while MMP2 transcription remained unchanged [52].

Kai1

Kai1 (also known as CD82 or C33) is a member of the tetraspanin superfamily (TM4SF) of adhesion molecules and was recently discovered as a prostate cancer metastasis-suppressor gene mapping to chromosome 11p11.2-p13 [53;54]. Kai1, like other members of the TM4SF family has been associated with metastatic potential of non small-cell human lung, liver, pancreatic bladder, breast, prostate and esophageal carcinomas and melanomas [reviewed in [55]]. Down-regulation of the *KAI1* is observed during the progression of human prostatic cancer, but mutations or allelic loss do not appear to be the major means for alteration.[56]. Mechanisms in other tumor types have not been so extensively evaluated.

The role of Kai1 in breast cancer metastasis has been implicated by several studies. Kai1 mRNA expression progressively decreased in a panel of human cell lines representing a continuum from nearly normal breast cells (MCF10A) to highly metastatic cells (MDA-MB-435) [57]. Transfection of Kai1 into

MDA-MB-435 cells suppressed metastasis from the mammary fat pad [25]. However, the cell lines did not maintain transgene expression levels following *in vivo* growth.

The mechanism of action of Kai1 is not completely understood. Several preliminary reports suggest that expression of Kai1 decreases the both the invasiveness and motility of cells in vitro [58;59]. These studies also showed that KAI1 transfectants exhibited enhanced Ca^{++}-independent aggregation suggesting that KAI1 might alter cell-cell interactions.

E-cadherin

E-cadherin is a cell surface glycoprotein involved in calcium-dependent cell-cell adhesion. Reduced levels of E-cadherin are associated with decreased adhesion and increased grade of epithelial neoplasms while increased E-cadherin expression (induced by transfection) decreases motility and invasiveness [60]. Mutations of E-cadherin and the associated protein α-catenin have been associated with acquisition of the invasive phenotype [61]. High E-cadherin levels inhibit shedding of tumor cells from the primary tumor; thus, E-cadherin is considered a metastasis-suppressor [26;61-64]. However, there is also evidence that it can function as a tumor suppressor gene [27;61;62].

A specific role for E-cadherin in breast cancer progression has not yet been established. However, mutations were detected, using PCR single strand conformation polymorphism assays, in lobular carcinomas [65;66]. Interestingly, infiltrating ductal and medullary breast carcinomas showed few mutations. This highlights the point made above about lumping all tumors together.

It has even been suggested that E-cadherin function could be restored by treatment with Tamoxifen [67], but whether this takes place in a clinical setting has not been explored to our knowledge.

BRMS1

Introduction of a normal, neo-tagged, human chromosome 11 into MDA-MB-435 suppressed metastasis without affecting tumorigenicity [68], leading to the hypothesis that a metastasis-suppressor

gene(s) is encoded on chromosome 11. Differential display was performed to identify those genes and a novel gene, BRMS1 (**Breast Metastasis Suppressor 1**) was cloned [69]. Transfection into MDA-MB-435 and MDA-MB-231 breast carcinoma cell lines suppressed metastasis without affecting tumorigenicity in a mouse model. The gene mapped to 11q13.1-q13.2, a region frequently altered in late-stage breast carcinoma. Following transfection, BRMS1 restored gap junctional intercellular communication between cells whereas vector-only transfectants still did not communicate in this manner. BRMS1 transfectants were also significantly suppressed for motility *in vitro* . No data regarding expression or mutation patterns in human cancers yet exists.

MKK4

The introduction of a discontinuous ~70 cM portion of human chromosome 17 significantly suppresses the metastatic ability of AT6.1 rat prostate cancer cells without affecting tumorigenicity [18]. AT6.1 cells containing the ~ 70 cM region escape from the primary tumor and arrest in the lung but are growth inhibited unless the metastasis-suppressor region is lost [18]. A combined differential expression and candidate gene approach identified the mitogen-activated protein kinase kinase 4/stress-activated protein/Erk kinase 1 (*MKK4/SEK1*) as a candidate metastasis-suppressor gene encoded by the ~ 70 cM region [29]. Transfection of an *MKK4/SEK1* expression construct significantly suppressed metastasis without affecting primary tumor growth. *In vivo* studies showed that AT6.1 cells expressing the *MKK4/SEK1* transgene recapitulate the dormant phenotype conferred by the ~70 cM region of chromosome 17 [29].

Previous studies had identified *MKK4/SEK1* as a candidate tumor suppressor gene [reviewed in [29]]. These studies identified homozygous deletions and other inactivating mutations in *MKK4/SEK1* in a small percentage of lung, pancreatic, and breast cancer cell lines and/or xenografts. Importantly, *MKK4/SEK1* can be an independent target for LOH – i.e., its inactivation is not just a by-product of large deletions of the nearby p53 gene. Recent studies using transgenic approaches found that disruption of the *MKK4/SEK1* gene caused embryonic death in mice, demonstrating a requirement for *MKK4/SEK1* in

development [reviewed in [29]]. These studies also included the analyses of cells with a homozygous deficiency in *MKK4/SEK1* and demonstrated that it is required for the normal regulation of cellular responses to environmental stress.

Colonization of the secondary site and future directions

The purpose of all the research highlighted in this review has been to improve cure rates and patient quality of life. It has been argued that agents that prevent metastasis will be meaningless since the "horse will have already escaped the barn." Administration of a preventative agent for an event that has occurred prior to diagnosis would indeed be useless. However, for inoperative lesions in an adjuvant setting, metastasis prevention may have a role.

Attention, then, turns to treatment of established metastases. In essence, *all* non-surgical cancer therapy currently in use is essentially for this purpose. Once a tumor is removed, any additional treatment is aimed at eliminating microscopic or detectable systemic disease. What limits the current approaches is tumor heterogeneity, plasticity of the tumor cell response and ineffective drug targeting.

How then will understanding the genetic basis of metastasis overcome these limitations? The answer is alluded in some recent studies from our laboratories. In short, independently-discovered metastasis suppressors for prostate carcinoma and melanoma both inhibit the formation of metastases by blocking growth at the secondary site [17;18]. In these studies, melanoma cells carrying chromosome 6 or rat prostatic carcinoma cells carrying chromosome 17 were tagged and followed in spontaneous metastasis assays in mice. For instance, chromosome 17 expressing, tagged cells were found as microscopic metastases in the lungs at rates comparable to the number of detectable metastases produced by the metastatic parental cell line. The chromosome 17 expressing, tagged cells could be retrieved from the lungs and expanded in culture, demonstrating their vitality. No evidence was found for anti-angiogenesis by the chromosome 17 hybrid cells, indicating a lack of colonization (i.e,. not angiogenesis) as a primary mechanism. For the chromosome 6 expressing, tagged melanoma cells, *in vitro* explants of pulmonary

micrometastases were actually injected into mice at an orthotopic site. The mice developed tumors which grew at a rate similar to the original cell line, further demonstrating that growth in the primary and secondary sites are, to some degree, differentially regulated. In other words, the metastasis-suppressed cells complete every step of the metastatic cascade prior to proliferation at the secondary site to form macroscopic metastases.

Although the existence of control mechanisms at this step of the metastatic cascade have long been inferred based upon logic, these data are the first hints at a molecular target. Neither of these genes could have been discovered without studying the *entire* metastatic process since no *in vitro* assays yet recapitulate metastasis. Indeed, our laboratories are working hard to develop such assays. Nonetheless, these results show that *in vivo* assays still have a role and that novel, interesting and potentially clinically relevant genes can be discovered using them.

A second example of impaired colonization potentially applicable to growth at a secondary site is found in the Nm23 literature. Colonization of Nm23-transfected K1735 melanoma and MDA-MB-435 human breast carcinoma cells in soft agar was reduced as compared to control transfectants, despite the observations among all transfection studies reported to date that primary tumor sizes are equivalent.

The cytokine TGFβ has been reported to be inhibitory to cell growth of many normal cells, but recently has been widely reported to stimulate growth or colonization of more advanced or metastatically competent cells [reviewed in [70;71]]. Addition of TGFβ to soft agar cultures of control- and Nm23-transfected cells recapitulated this trend: TGF-β stimulated several-fold the colonization of metastatic, control transfectants, but was generally without effect on the Nm23 transfectants [33;72]. It is hypothesized that, in a secondary site, where locally produced growth factors and cell-cell interactions may be different than those at the primary site, the cancer cells, which can utilize a widely available growth factor such as TGFβ as a stimulant, would have a metastatic advantage. Other cytokines, such as IL-6, have also been reported to exhibit a similar switch to the stimulation of aggressive cancer cells. The mechanism of the TGFβ "switch" is unknown, but of potential translational relevance. Investigations in

other model systems have identified TGFβ-induced alterations in cell-cell interactions in the liver [73], production of anti-apoptotic proteins [74], enhancement of proteinase activity [75;76], and induction of angiogenic factor production [77]. In bone metastasis, TGF-β is produced by osteoclasts and induces parathyroid hormone related protein production by tumor cells in a positive feedback loop [78;79]. Other non-TGF-β-related studies of colonization also point to a myriad of potential control points. The most amazing aspect of this list is its overlap with the regulation of more traditionally studied aspects of metastasis – adhesion, proteolysis, motility.

Colonization in various models has been influenced by adhesion proteins such as CD44 [80], α6 integrins [81;82], galectin-3 [83], lung dipeptidyl peptidase [84] and N-cadherin [85], the latter pointing not only to adherence but a cellular epithelial-mesenchymal transition [86]. The target of much adhesion, the stroma or extracellular matrix, is also reported in the colonization literature. Proteinases, such as matrix metalloproteinases and plasminogen activators, have been implicated in colonization, not only to include degradation but effects on tumor dormancy [87;88]. Growth factors and their receptors such as c-met and IGF receptor have been implicated in colonization [89;90]. Importantly, overexpression of FGF in MCF7 breast carcinoma cells facilitated dissemination from the primary tumor but not lung colonization [91], showing that not any factor can be assigned to this phenotype. Potential colonization regulatory points outside of the traditional invasion arena include apoptosis and angiogenesis [92;93].

These data imply that whatever gene(s) and protein(s) are responsible could be exploited at two levels in the clinic. First, a mimetic could be used to prevent establishment of new metastases. This is demonstrated by the pre-clinical studies performed using animal models. Second, a mimetic could block growth of the metastases and, perhaps, even casue the metastases to regress. The data also imply that many of the traditional components of metastasis research could be relevant to the study of colonization at the secondary site. They also highlight the need for better models.

The genetics of metastasis in general, and breast cancer specifically, is complex and still poorly understood. While new genes/proteins are being identified at an increasingly rapid rate, a comprehensive

and unifying model for interactions between them will require more research.

Acknowledgments: We apologize to the many authors whose work was not cited due to space limitations. Several reviews are listed as "of importance" and "of significant importance" because they more completely review details of aspects of the metastatic process than are possible in this format.

References:

1. American Cancer Society: **Cancer Statistics 1998**. *CA Cancer J.Clin.* 1998, **48**:1-63.

2. Boyd D: **Invasion and metastasis** . *Cancer Metastasis Rev.* 1996, **15**:77-89.

3. Price JT, Bonovich MT, Kohn EC: **The biochemistry of cancer dissemination**. *Crit.Rev.Biochem.Mol.Biol.* 1997, **32**:175-253.

   • This is an up-to-date, thorough review describing the multitude of molecules implicated in the metastatic process.

4. Ruoslahti E: **How cancer spreads** . *Sci.Am.* 1996, **275**:72-77.

5. Welch DR: **Technical considerations for studying cancer metastasis *in vivo***. *Clin.Exptl.Metastasis* 1997, **15**:272-306.

6. Zetter BR: **Angiogenesis and tumor metastasis**. *Annu.Rev.Med.* 1998, **49**:407-424.

7. Chambers AF: **The metastatic process: basic research and clinical implications**. *Oncol.Res.* 1999, **11**:161-168.

8. Paget S: **The distribution of secondary growths in cancer of the breast**. *Lancet* 1889, **1**:571-573.

   • The first systematic comparison of the distribution of metastasis in human cancer. This paper was the first to formally recognize the integral relationship between tumor and host cells in the process of metastasis.

9. Nicolson GL: **Cancer metastasis: tumor cell and host organ properties important in metastasis to secondary sites** . *Biochim.Biophys.Acta* 1988, **948**:175-224.

10. Nicolson GL: **Paracrine and autocrine growth mechanisms in tumor metastasis to specific sites with particular emphasis on brain and lung metastasis**. *Cancer Metastasis Rev.* 1993, **12**:235-343.

11. Nicolson GL: **Cancer progression and growth: relationship of paracrine and autocrine growth mechanisms to organ preference of metastasis**. *Exp.Cell Res.* 1993, **204**:171-180.

12. Zetter BR: **Cellular basis of site-specific tumor metastasis.** *N.Engl.J.Med.* 1990, **322:**605-612.

13. Radinsky R: **Modulation of tumor cell gene expression and phenotype by the organ specific metastatic environment.** *Cancer Metastasis Rev.* 1995, **14:**323-338.

14. Pfeifer JD, Wick MR: **The pathologic evaluation of neoplastic diseases.** In *Clinical Oncology*, edn 2nd. Edited by Murphy GP, Lawrence W, Lenhard RE. Atlanta: American Cancer Society; 1995:75-95.

15. Welch DR, Rinker-Schaeffer CW: **What defines a useful marker of metastasis in human cancer?** *J.Natl.Cancer Inst.* 1999, **91:**1351-1353.

16. Fidler IJ, Radinsky R: **Genetic control of cancer metastasis.** *J.Natl.Cancer Inst.* 1990, **82:**166-168.

17. Goldberg SF, Harms JF, Quon K, Welch DR: **Metastasis-suppressed C8161 melanoma cells arrest in lung but fail to proliferate.** *Clin.Exptl.Metastasis* 2000, **(in press).**

     •• Examines the mechanism of metastasis suppression in human melanoma cells following introduction of chromosome 6. Green fluorescent protein-tagged cells are observed in the lungs following injection, but fail to proliferate. However, the same cells removed from the lungs grow in an orthotopic site.

18. Chekmareva MA, Kadkhodaian MM, Hollowell CMP, Kim H, Yoshida BA, Luu HH, Stadler WM, Rinker-Schaeffer CW: **Chromosome 17-mediated dormancy of AT6.1 prostate cancer micrometastases.** *Cancer Res.* 1998, **58:**4963-4969.

     •• Shows that metastasis-suppressed prostate carcinoma cells are able to leave the primary tumor and colonize lung, but the colonies fail to proliferate.

19. Welch DR, Wei LL: **Genetic and epigenetic regulation of human breast cancer progression and metastasis.** *Endocrine-related Cancer* 1998, **5:**155-197.

     •• This review surveys several thousand papers and summarizes the genes and chromosomal abnormalities implicated in the development and progression of breast carcinoma.

20. Freije JMP, MacDonald NJ, Steeg PS: **Differential gene expression in tumor metastasis: Nm23.**

Curr.Topics Microbiol.Immunol. 1996, **213:**215-232.

• A comprehensive review of the basic and clinical data involving the Nm23 metastasis suppressor gene.

21. Weinstat-Saslow DL, Steeg PS: **Angiogenesis and colonization in the tumor metastatic process: Basic and applied advances.** *FASEB J.* 1994, **8:**401-407.

22. Lee J-H, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, Welch DR: ***KiSS-1,* a novel human malignant melanoma metastasis-suppressor gene.** *J.Natl.Cancer Inst.* 1996, **88:**1731-1737.

• Describes the isolation and initial characterization of the KiSS1 metastasis suppressor gene.

23. Lee J-H, Welch DR: **Identification of highly expressed genes in metastasis-suppressed chromosome 6/human malignant melanoma hybrid cells using subtractive hybridization and differential display.** *Intl.J.Cancer* 1997, **71:**1035-1044.

24. Dong J-T, Lamb PW, Rinker-Schaeffer CW, Vukanovic J, Ichikawa T, Isaacs JT, Barrett JC: ***KAI1,* a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2.** *Science (Wash.D.C.)* 1995, **268:** 884-886.

• Describes the discovery of Kai1 as a metastasis suppressor gene in prostate carcinoma and implicates it (and other tetraspan family members) in controlling metastasis by several cancer types.

25. Phillips KK, White AE, Hicks DJ, Welch DR, Barrett JC, Wei LL, Weissman BE: **Correlation between reduction of metastasis in the MDA-MB-435 model system and increased expression of the Kai-1 protein.** *Molec.Carcinog.* 1998, **21:**111-120.

26. Perl AK, Wilgenbus P, Dahl U, Semb H, Christofori G: **A causal role for E-cadherin in the transition from adenoma to carcinoma.** *Nature (London)* 1998, **392:**190-193.

• Demonstrates, using a series of elegant experiments involving dominant negative constructs in RipTag transgenic mice, that E-cadherin is involved in the conversion of pancreatic carcinoma cells to the invasive phenotype.

27. Christofori G, Semb H: **The role of the cell-adhesion molecule E-cadherin as a tumour- suppressor gene.** *Trends Biochem.Sci.* 1999, **24**:73-76.

28. Samant RS, Seraj MJ, Meehan WJ, Harms JF, Leonard TO, Shevde LA, Sakamaki T, Winter CR, Verderame MF, Welch DR: **BrMS1 — a human breast carcinoma metastasis-suppressor gene [abstract].** *Proc.Am.Assoc.Cancer Res.* 2000, **41**:1053.

29. Yoshida BA, Dubauskas Z, Chekmareva MA, Christiano TR, Stadler WM, Rinker-Schaeffer CW: **Mitogen-activated protein kinase kinase 4/stress-activated protein/Erk kinase 1 (*MKK4/SEK1*), a prostate cancer metastasis suppressor gene encoded by human chromosome 17.** *Cancer Res.* 1999, **59**:5483-5487.

   • Provides evidence that MKK4 is a metastasis suppressor gene in prostate carcinoma.

30. Freije JM, MacDonald NJ, Steeg PS: **Nm23 and tumour metastasis: basic and translational advances.** *Biochem.Soc.Symp.* 1998, **63**:261-271.

31. Kapranos N, Karaiossifidi H, Kouri E, Vasilaros S: **Nm23 expression in breast ductal carcinomas: a ten year follow-up study in a uniform group of node-negative breast cancer patients.** *Anticancer Res.* 1996, **16**:3987-3990.

32. Russell RL, Geisinger KR, Mehta RR, White WL, Shelton B, Kute TE: **nm23 - Relationship to the metastatic potential of breast carcinoma cell lines, primary human xenografts, and lymph node negative breast carcinoma patients.** *Cancer* 1997, **79**:1158-1165.

33. Leone A, Flatow U, VanHoutte K, Steeg PS: **Transfection of human nm23-H1 into the human MDA-MB-435 breast carcinoma cell line: effects on tumor metastatic potential, colonization and enzymatic activity.** *Oncogene* 1993, **8**:2325-2333.

34. Postel EH: **NM23-NDP kinase.** *Int.J.Biochem.Cell Biol.* 1998, **30**:1291-1295.

35. De La Rosa A, Williams RL, Steeg PS: **Nm23/nucleoside diphosphate kinase: Toward a structural and biochemical understanding of its biological functions.** *BioEssays* 1995, **17**:53-62.

36. MacDonald NJ, De La Rosa A, Benedict MA, Freije JM, Krutsch H, Steeg PS: **A serine phosphorylation of**

Nm23, and not its nucleoside diphosphate kinase activity, correlates with suppression of tumor metastatic potential. *J.Biol.Chem.* 1993, **268**:25780-25789.

37. Cipollini G, Berti A, Fiore L, Rainaldi G, Basolo F, Merlo G, Bevilacqua G, Caligo MA: **Down-regulation of the Nm23-H1 gene inhibits cell proliferation.** *Int.J.Cancer* 1997, **73**:297-302.

38. Freije JM, Blay P, MacDonald NJ, Manrow RE, Steeg PS: **Site-directed mutation of Nm23-H1. Mutations lacking motility suppressive capacity upon transfection are deficient in histidine-dependent protein phosphotransferase pathways in vitro.** *J.Biol.Chem.* 1997, **272**:5525-5532.

39. Wagner PD, Vu ND: **Histidine to aspartate phosphotransferase activity of nm23 proteins: phosphorylation of aldolase C on Asp-319.** *Biochemical Journal* 2000, **346**:623-630.

40. Postel EH, Berberich SJ, Flint SJ, Ferrone CA: **Human c-*myc* transcription factor PuF identified as nm23-H2 nucleoside diphosphate kinase, a candidate suppressor of tumor metastasis.** *Science (Wash.D.C.)* 1993, **261**:478-480.

41. Engel M, Theisinger B, Seib T, Seitz G, Huwer H, Zang KD, Welter C, Dooley S: **High levels of nm23-H1 and nm23-H2 messenger RNA in human squamous cell lung carcinoma are associated with poor differentiation and advanced tumor stages.** *Intl.J.Cancer* 1993, **55**:375-379.

42. Mandai M, Konishi I, Koshiyama M, Mori T, Arao S, Tashiro H, Okamura H, Nomura H, Hiai H, Fukumoto M: **Expression of metastasis-related *nm23-H1* and *nm23-H2* genes in ovarian carcinomas: Correlation with clinicopathology, *EGFR*, c-*erb*B-2, and c-*erb*B-3 genes, and sex steroid receptor expression.** *Cancer Res.* 1994, **54**:1825-1830.

43. Marone M, Scambia G, Ferrandina G, Giannitelli C, Benedetti-Panici P, Iacovella S, Leone A, Mancuso S: **Nm23 expression in endometrial and cervical cancer: Inverse correlation with lymph node involvement and myometrial invasion.** *Br.J.Cancer* 1996, **74**:1063-1068.

44. Arai T, Watanabe M, Onodera M, Yamashita T, Masunaga A, Itoyama S, Itoh K, Sugawara I: **Reduced nm23-H1 messenger RNA expression in metastatic lymph nodes from patients with papillary carcinoma of the thyroid.** *Am.J.Pathol.* 1993, **142**:1938-1944.

45. Baba H, Urano T, Okada K, Furukawa K, Nakayama E, Tanaka H, Iwasaki K, Shiku H: **Two isotypes of** murine *nm23*/**nucleoside diphosphate kinase,** *nm23-M1* **and** *nm23-M2,* **are involved in metastatic suppression of a murine melanoma line**. *Cancer Res.* 1995, **55**:1977-1981.

46. Tokunaga Y, Urano T, Furukawa K, Kondo H, Kanematsu T, Shiku H: **Reduced expression of** *nm23*-**H1, but not of** *nm23*-**H2, is concordant with the frequency of lymph node metastasis of human breast cancer**. *Intl.J.Cancer* 1993, **55**:66-71.

47. Yamaguchi A, Urano T, Goi T, Takeuchi K, Niimoto S, Nakagawara G, Furukawa K, Shiku H: **Expression of human** *nm*23-**H1 and** *nm*23-**H2 proteins in hepatocellular carcinoma**. *Cancer* 1994, **73**:2280-2284.

48. Welch DR, Chen P, Miele ME, McGary CT, Bower JM, Weissman BE, Stanbridge EJ: **Microcell-mediated transfer of chromosome 6 into metastatic human C8161 melanoma cells suppresses metastasis but does not inhibit tumorigenicity**. *Oncogene* 1994, **9**:255-262.

49. Miele ME, Robertson G, Lee JH, Coleman A, McGary CT, Fisher PB, Lugo TG, Welch DR: **Metastasis suppressed, but tumorigenicity and local invasiveness unaffected, in the human melanoma cell line MelJuSo after introduction of human chromosomes 1 or 6**. *Molec.Carcinog.* 1996, **15**:284-299.

50. West A, Vojta PJ, Welch DR, Weissman BE: **Chromosome localization and genomic structure of the KiSS-1 metastasis suppressor gene (KISS1)**. *Genomics* 1998, **54**:145-148.

51. Lee J-H, Welch DR: **Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene, KiSS-1**. *Cancer Res.* 1997, **57**:2384-2387.

52. Yan C, Boyd D: **Down-regulation of 92 kDa type IV collagenase expression by the metastasis suppressor gene KiSS-1**. *Proc.Am.Assoc.Cancer Res.* 2000, **41**:3486.

53. Dong JT, Isaacs WB, Barrett JC, Isaacs JT: **Genomic organization of the human KAI1 metastasis-suppressor gene**. *Genomics* 1997, **41**:25-32.

54. Kawana Y, Komiya A, Ueda T, Nihei N, Kuramochi H, Suzuki H, Yatani R, Imai T, Dong JT, Yoshie O, Barrett JC, Isaacs JT, Shimazaki J, Ito H, Ichikawa T: **Location of KAI1 on the short arm of human**

chromosome 11 and frequency of allelic loss in advanced human prostate cancer. *Prostate* 1997, **32**:205-213.

55. Dong JT, Isaacs WB, Isaacs JT: **Molecular advances in prostate cancer**. *Curr.Opin.Oncol.* 1997, **9**:101-107.

56. Dong JT, Suzuki H, Pin SS, Bova GS, Schalken JA, Isaacs WB, Barrett JC, Isaacs JT: **Down-regulation of the *KAI1* metastasis suppressor gene during the progression of human prostatic cancer infrequently involves gene mutation or allelic loss**. *Cancer Res.* 1996, **56**:4387-4390.

57. Yang XH, Welch DR, Phillips KK, Weissman BE, Wei LL: **KAI1, a putative marker for metastatic potential in human breast cancer**. *Cancer Lett.* 1997, **119**:149-155.

58. Takaoka A, Hinoda Y, Satoh S, Adachi Y, Itoh F, Adachi M, Imai K: **Suppression of invasive properties of colon cancer cells by a metastasis suppressor KAI1 gene**. *Oncogene* 1998, **16**:1443-1453.

59. Takaoka A, Hinoda Y, Sato S, Itoh F, Adachi M, Hareyama M, Imai K: **Reduced invasive and metastatic potentials of KAI1-transfected melanoma cells**. *Jpn.J.Cancer Res.* 1998, **89**:397-404.

60. Mareel M, Boterberg T, Noe V, Van Hoorde L, Vermeulen S, Bruyneel E, Bracke M: **E-cadherin/catenin/cytoskeleton complex: A regulator of cancer invasion**. *J.Cell Physiol.* 1997, **173**:271-274.

61. Vermeulen SJ, Bruyneel EA, Bracke ME, De Bruyne GK, Vennekens KM, Vleminckx KL, Berx GJ, Van Roy FM, Mareel MM: **Transition from the noninvasive to the invasive phenotype and loss of α-catenin in human colon cancer cells**. *Cancer Res.* 1995, **55**:4722-4728.

62. Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Lochner D, Birchmeier W: **E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells**. *J.Cell Biol.* 1991, **113**:173-185.

63. Mbalaviele G, Dunstan CR, Sasaki A, Williams PJ, Mundy GR, Yoneda T: **E-cadherin expression in human breast cancer cells suppresses the development of osteolytic bone metastases in an experimental

metastasis model. *Cancer Res.* 1996, **56:**4063-4070.

64. Oka H, Shiozaki H, Kobayashi K, Inoue M, Tahara H, Kobayashi T, Takatsuka Y, Matsuzaki N, Hirano S, Takeichi M: **Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis.** *Cancer Res.* 1993, **53:**1696-1701.

65. Berx G, Cleton-Jansen AM, Nollet F, De Leeuw WJF, Van de Vijver MJ, Cornelisse CJ, Van Roy F: **E-cadherin is a tumour invasion suppressor gene mutated in human lobular breast cancers.** *EMBO J.* 1995, **14:**6107-6115.

66. Berx G, Cleton-Jansen AM, Strumane K, De Leeuw WJF, Nollet F, Van Roy F, Cornelisse C: **E-cadherin is inactivated in a majority of invasive human lobular breast cancers by truncation mutations throughout its extracellular domain.** *Oncogene* 1996, **13:**1919-1925.

67. Bracke ME, Charlier C, Bruyneel EA, Labit C, Mareel MM, Castronovo V: **Tamoxifen restores the E-cadherin function in human breast cancer MCF-7/6 cells and suppresses their invasive phenotype.** *Cancer Res.* 1994, **54:**4607-4609.

68. Phillips KK, Welch DR, Miele ME, Lee J-H, Wei LL, Weissman BE: **Suppression of MDA-MB-435 breast carcinoma cell metastasis following the introduction of human chromosome 11.** *Cancer Res.* 1996, **56:**1222-1226.

69. Seraj MJ, Samant RS, Verderame MF, Welch DR. Functional evidence for a novel human breast carcinoma metastasis suppressor, *BRMS1*, encoded at chromosome 11q13. Cancer Research 2000 (in press).

70. Padgett RW, Das P, Krishna S: **TGF-beta signaling, Smads, and tumor suppressors.** *BioEssays* 1998, **20:**382-390.

71. Hartsough MT, Mulder KM: **Transforming growth factor-beta signaling in epithelial cells.** *Pharmacol. Ther.* 1997, **75:**21-41.

72. Leone A, Flatow U, King CR, Sandeen MA, Margulies IMK, Liotta LA, Steeg PS: **Reduced tumor incidence, metastatic potential, and cytokine responsiveness of nm23-transfected melanoma cells.** *Cell*

1991, **65**:25-35.

73. Nishibori H, Watanabe M, Narai S, Kubota T, Matsubara C, Teramoto T, Kitajima M: **An interaction between human colon carcinoma cells and hepatocytes activates transforming growth factor-beta1 in vitro**. *Cancer Lett.* 1999, **142**:83-89.

74. Carey GB, Chang NS: **Cloning and characterization of a transforming growth factor beta 1- induced anti-apoptotic adhesion protein TIF2**. *Biochem.Biophys.Res.Commun.* 1998, **249**:283-286.

75. Welch DR, Fabra A, Nakajima M: **Transforming growth factor beta stimulates mammary adenocarcinoma cell invasion and metastatic potential**. *Proc.Natl.Acad.Sci.(USA)* 1990, **87**:7678-7682.

76. Duivenvoorden WCM, Hirte HW, Singh G: **Transforming growth factor β1 acts as an inducer of matrix metalloproteinase expression and activity in human bone-metastasizing cancer cells**. *Clin.Exptl.Metastasis* 1999, **17**:27-34.

77. Donovan D, Harmey JH, Toomey D, Osborne DH, Redmond HP, Bouchier-Hayes DJ: **TGF beta-1 regulation of VEGF production by breast cancer cells [see comments]**. *Ann.Surg.Oncol.* 1997, **4**:621-627.

78. Yin JJ, Selander K, Chirgwin JM, Dallas M, Grubbs BG, Wieser R, Massagué J, Mundy GR, Guise TA: **TGF-β signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development**. *J.Clin.Invest.* 1999, **103**:197-206.

79. Guise TA: **Parathyroid hormone-related protein and bone metastases**. *Cancer* 1997, **80**:1572-1580.

80. Kogerman P, Sy MS, Culp LA: **Overexpressed human CD44s promotes lung colonization during micrometastasis of murine fibrosarcoma cells: Facilitated retention in the lung vasculature**. *Proc.Natl.Acad.Sci.USA* 1997, **94**:13233-13238.

81. Mukhopadhyay R, Theriault RL, Price JE: **Increased levels of α6 integrins are associated with the metastatic phenotype of human breast cancer cells**. *Clin.Exptl.Metastasis* 1999, **17**:325-332.

82. Wewer UM, Shaw LM, Albrechtsen R, Mercurio AM: **The integrin α6β1 promotes the survival of**

metastatic human breast carcinoma cells in mice. *Am.J.Pathol.* 1997, **151**:1191-1198.

83. Ochieng J, Warfield P, Green-Jarvis B, Fentie I: **Galectin-3 regulates the adhesive interaction between breast carcinoma cells and elastin.** *J.Cell.Biochem.* 1999, **75**:505-514.

84. Cheng HC, Abdel-Ghany M, Elble RC, Pauli BU: **Lung endothelial dipeptidyl peptidase IV promotes adhesion and metastasis of rat breast cancer cells via tumor cell surface-associated fibronectin.** *J.Biol.Chem.* 1998, **273**:24207-24215.

85. Tran NL, Nagle RB, Cress AE, Heimark RL: **N-Cadherin expression in human prostate carcinoma cell lines. An epithelial-mesenchymal transformation mediating adhesion with stromal cells.** *Am.J.Pathol.* 1999, **155**:787-798.

86. Hendrix MJC, Seftor EA, Chu YW, Trevor KT, Seftor REB: **Role of intermediate filaments in migration, invasion and metastasis.** *Cancer Metastasis Rev.* 1996, **15**:507-525.

87. Eccles SA, Box GM, Court WJ, Bone EA, Thomas W, Brown PD: **Control of lymphatic and hematogenous metastasis of a rat mammary carcinoma by the matrix metalloproteinase inhibitor batimastat (BB-94).** *Cancer Res.* 1996, **56**: 2815-2822.

88. Ossowski L, Ghiso JA, Liu D, Yu W, Kovalski K: **The role of plasminogen activator receptor in cancer invasion and dormancy [abstract].** *Medicina (B Aires)* 1999, **59**:547-552.

89. Lang SH, Clarke NW, George NJR, Testa NG: **Scatter factor influences the formation of prostate epithelial cell colonies on bone marrow stroma *in vitro*.** *Clin.Exptl.Metastasis* 1999, **17**:333-340.

90. Long L, Rubin R, Brodt P: **Enhanced invasion and liver colonization by lung carcinoma cells overexpressing the type 1 insulin-like growth factor receptor.** *Exp.Cell Res.* 1998, **238**:116-121.

91. Zhang L, Kharbanda S, McLeskey SW, Kern FG: **Overexpression of fibroblast growth factor 1 in MCF-7 breast cancer cells facilitates tumor cell dissemination but does not support the development of macrometastases in the lungs or lymph nodes.** *Cancer Res.* 1999, **59**:5023-5029.

92. Schwarz MA, Kandel J, Brett J, Li J, Hayward J, Schwarz RE, Chappey O, Wautier JL, Chabot J, Lo GP,

Stem D: **Endothelial-monocyte activating polypeptide II, a novel antitumor cytokine that suppresses primary and metastatic tumor growth and induces apoptosis in growing endothelial cells.** *J.Exp.Med.* 1999, **190:**341-354.

93.   Reed JC: **Dysregulation of apoptosis in cancer.** *J.Clin.Oncol.* 1999, **17:**2941-2953.

Advances in Brief

Functional Evidence for a Novel Human Breast Carcinoma Metastasis Suppressor, *BRMS1*, Encoded at Chromosome 11q13[1]

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Abstract

We previously showed that introduction of a normal, neomycin-tagged human chromosome 11 reduces the metastatic capacity of MDA-MB-435 (435) human breast carcinoma cells by 70–90% without affecting tumorigenicity, suggesting the presence of one or more metastasis suppressor genes encoded on human chromosome 11. To identify the gene(s) responsible, differential display comparing chromosome 11-containing (neo11/435) and parental, metastatic cells was done. We describe the isolation and functional characterization of a full-length cDNA for one of the novel genes, designated breast-cancer metastasis suppressor 1 (*BRMS1*), which maps to human chromosome 11q13.1-q13.2. Stably transfected MDA-MB-435 and MDA-MB-231 breast carcinoma cells still form progressively growing, locally invasive tumors when injected into mammary fat pads but are significantly less metastatic to lungs and regional lymph nodes. These data provide compelling functional evidence that breast-cancer metastasis suppressor 1 is a novel mediator of metastasis suppression in human breast carcinoma.

INTRODUCTION

When breast carcinoma cells are confined to breast tissue, long-term survival rates are high. But when tumor cells disseminate to and colonize secondary sites, cure rates drop significantly. Likewise, quality of life for patients with stage IV (metastatic) disease is significantly worse than for those with stage I (local) carcinoma. Thus, decreased morbidity and mortality will depend on prevention and/or effective treatment of metastatic disease. To that end, understanding the biological, biochemical, and genetic mechanisms underpinning tumor cell invasion and metastasis will be required.

Metastasis-regulatory genes can be broadly categorized as either metastasis-promoting or metastasis-suppressing. Analogous to the role of oncogenes in tumorigenesis, metastasis promoters drive conversion from nonmetastatic to metastatic. Although similar in other respects, metastasis suppressors are distinguishable from tumor suppressors in that the former block only metastasis when introduced into metastatic tumor cells (*i.e.*, not tumorigenicity). As expected, tumor suppressors suppress both phenotypes because tumorigenicity is a prerequisite to metastasis (1). To date, only six metastasis suppressor genes (*Nm23, KISS1, Kai1, E-cadherin, MKK4, TIMPs*) have been shown to functionally suppress metastasis using *in vivo* models (reviewed in Ref. 2).

Two general approaches were used to identify these metastasis-controlling genes. The first involved comparison of gene expression in poorly or nonmetastatic cells with matched metastasis-competent cells. The second took advantage of clinical observations that identified nonrandom chromosomal changes that occur during tumor progression. This information localized the gene(s) from which cloning could commence. In this study, we combined aspects of both strategies to identify a novel, functional breast carcinoma metastasis suppressor gene.

A recent cataloging of differential gene/protein gene expression and chromosomal abnormalities occurring as breast carcinoma acquires metastatic potential (2) revealed that some karyotypic changes commonly occur in early-stage breast cancer (8p, 13q, 16q, 17p, 17q, and 19p), whereas others typically occur later in breast cancer progression (1p, 1q, 3p, 6q, 7q, 11p, and 11q). Among the most common changes in both familial or sporadic breast carcinoma are losses of genetic material on chromosome 11q, which occurs in 40–65% of cases. There are several regions spanning the q-arm of chromosome 11 for which associations have been made with breast cancer progression. Among the most common are amplifications and deletions involving regions near band 11q13. Within this region, there is evidence supporting the existence of a number of critical genes, including tumor-promoting, tumor-suppressing, metastasis-promoting and metastasis-suppressing genes. The genes *int-2, hst, bcl-1, glutathione S-transferase, CCND1,* and *EMS-1*, which map to 11q13, are amplified in breast cancer at a frequency between 3 and 20%. There exists a high-frequency involvement of rat chromosome 1 (which is syntenic to human chromosome 11) in the development and progression of rat mammary tumors (3). Therefore, based on these observations and high-frequency deletions involving 11q13-q14 in late-stage, metastatic breast carcinomas, we tested the hypothesis that chromosome 11q encodes a metastasis suppressor gene. Upon finding that introduction of a normal human chromosome 11 into metastatic MDA-MB-435 (435) human breast carcinoma suppressed metastasis without affecting tumorigenicity (4), we set out to identify the gene(s) responsible. We report here the isolation and functional characterization of a metastasis suppressor gene from this region.

MATERIALS AND METHODS

Cell Lines and Cell Culture. MDA-MB-435 and MDA-MB-231 are human estrogen receptor- and progesterone receptor-negative cell lines derived from metastatic (pleural effusion), infiltrating ductal breast carcinoma. Both cell lines form progressively growing tumors when injected into the mammary fat pads of immunocompromised mice. MDA-MB-435 cells develop macroscopic metastases in the lungs and regional lymph nodes by 10–12 weeks postinoculation, but rarely metastasize after direct injection into the lateral tail vein. The opposite pattern exists for 231 in athymic mice. MDA-MB-435 cell clones into which a normal, neomycin-tagged human chromosome 11 had been introduced by microcell-mediated transfer (designated neo11/435) are suppressed at least 75% for metastasis from the mammary fat pad (4).

$BRMS1^5$ transfectants were derived after transfection of full-length $BRMS1$ cDNA (see below) cloned into the constitutive mammalian expression vector, pcDNA3 (Invitrogen, San Diego, CA). All cell lines were cultured in a 1:1 (v/v) mixture of DMEM and Ham's F-12 medium supplemented with 5% fetal bovine serum (Atlanta Biologicals, Atlanta, GA), 1% nonessential amino acids, and 1.0 mM sodium pyruvate, but no antibiotics or antimycotics. Transfected cells and neo11/435 hybrids also received 500 μg/ml Geneticin (G-418; Life Technologies, Inc., Gaithersburg, MD). $BRMS1$-transfected 435 cells acquired an unexplained acute sensitivity to trypsin; therefore, cultures were thereafter passaged using 2 mM EDTA solution in calcium- and magnesium-free Dulbecco's PBS. Hybrid clones and transfectants were used before passage 11 in all cases to minimize the impacts of clonal diversification and phenotypic instability. For all functional and biological assays, cells between 70 and 90% confluence were used with viability >95%. All lines were routinely checked and found to be negative for *Mycoplasma* spp. contamination using the GenProbe method (Fisher Scientific, Pittsburgh, PA).

Cell line nomenclature was developed to identify the origin and nature of each cell line as unambiguously as possible. Single-cell clones are identified by the parental cell line name preceding a "." followed by a clonal designation. Uncloned populations are identified by a "−" after the parental cell line name. Microcell hybrids are identified by the tagged chromosome number followed by a "/" (e.g., neo11/435.A3 is single cell clone A3 derived after microcell-mediated transfer of chromosome 11 into MDA-MB-435). Where appropriate, numbers in parentheses following the cell line designation indicate the number of subcultures following cloning or establishment of the cell line. Numbers preceded by a "TE" indicate that the cells were passaged in a mixture of 0.125% trypsin-2 mM EDTA in calcium- and magnesium-free Dulbecco's PBS. Numbers preceded by a "P" indicate the cells were passaged using EDTA alone.

Transfections. $BRMS1$ was cloned into the constitutive mammalian expression vector, pcDNA3 (Invitrogen). To detect BRMS1 protein expression, a chimeric molecule was also constructed with an NH_2-terminal epitope tag (SV40T epitope 901; Ref. 5). Epitope-tagged and native $BRMS1$ plasmids as well as pcDNA3 vector only were transfected into 435 and 231 cells by electroporation. After selection in G-418, single-cell clones were isolated by limiting dilution. Stable transfectants were assessed for their expression by RNA blot and, as appropriate, by immunoblot.

DD-RTPCR. To identify differences in mRNA expression between metastatic and nonmetastatic neo11/435 hybrid cells, a DD-RTPCR approach was undertaken. The methods used involved modifications of the method described by Liang and Pardee (6) as available in the Delta Differential Display kit (Clontech Laboratories, Inc., Palo Alto, CA). To minimize the impact of clonal heterogeneity, a mixture of equal parts mRNA from three neo11/435 hybrid clones [neo11/435.A3 (TE5), neo11/435.B1 (TE4), and neo11/435.D1 (TE10)] were used. To reduce the chances of proceeding with irrelevant cDNAs, we validated findings at several intermediate steps during the experiment. In short, every step was replicated using independent samples. Once PCR products were validated, they were used as probes to examine differential expression in Northern blots using progressively more extensive series of cell-line mRNAs. As full-length cDNA clones were obtained, replicate Northern blots were done with those as well to verify prior results.

Differentially expressed cDNAs and the primers used for the initial DD-RTPCR reactions were as follows: $BRMS1$ (P9/P9); $F5A3$ (P9/T5), $8A3$ (P3/T4); adenine phosphoribosyltransferase (P1/T9); N-acetyl-galactosamine-6-sulfate sulfatase (P6/P6); hexokinase II (P10/T8):

P1: 5'-ATTAACCCTCACTAAATGCTGGGGA-3'
P3: 5'-ATTAACCCTCACTAAATGCTGGTGG-3'
P6: 5'-ATTAACCCTCACTAAATGCTGGGTG-3'
P9: 5'-ATTAACCCTCACTAAATGTGGCAGG-3'
P10: 5'-ATTAACCCTCACTAAAGCACCGTCC-3'
T4: 5'-CATTATGCTGAGTGATATCTTTTTTTTTTCA-3'
T5: 5'-CATTATGCTGAGTGATATCTTTTTTTTTTCC-3'
T8: 5'-CATTATGCTGAGTGATATCTTTTTTTTTTGC-3'
T9: 5'-CATTATGCTGAGTGATATCTTTTTTTTTTGG-3'

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[5] The abbreviations used are: $BRMS1$, breast cancer metastasis suppressor-1; DD-RTPCR, differential display; DAPI, 4',6-diamidino-2-phenylindole; RT-PCR, reverse transcription-PCR; EST, expressed sequence tags.

F5A3 and 8A3 were provisional nomenclature used during the initial part of these studies.

Chromosomal Localization of BRMS1. $BRMS1$ cDNA was used to screen bacterial artificial chromosome and P1 artificial chromosome libraries at Genome Systems, Inc. (St. Louis, MO). Bacterial artificial chromosome clones 412(n24) and 536(h18) harbored $BRMS1$ as confirmed by direct sequencing.[6] The genomic sequence was determined using the 412(n24) clone). DNA was isolated and labeled with digoxigenin dUTP by nick translation and combined with sheared human DNA before hybridization to metaphase chromosomes derived from phytohemagglutinin-stimulated peripheral blood leukocytes in a solution containing 50% formamide, 10% dextran sulfate, and 2× SSC. Specific hybridization signals were detected by exposing the hybrid cell lines to antidigoxigenin antibodies followed by counterstaining with DAPI. Specific labeling was seen along the proximal long arm of a group C chromosome, which was subsequently confirmed to be chromosome 11 based on cohybridization with genomic probes known to map to 11p15 and 11cen. Measurements of 71 of 80 specifically labeled chromosomes 11 in metaphase spreads demonstrated that $BRMS1$ is located at a position that is 19% of the distance from the centromere to the telomere of chromosome 11q. This corresponds to band 11q13.1-q13.2 (data not shown).

Metastasis Assays. Immediately prior to injection, cells (7–11 passages after transfection) at 80–90% confluence were detached with a 2 mM EDTA solution. Cells were washed, counted on a hemacytometer, and resuspended in ice-cold HBSS to a final concentration of 2.5×10^6 cells/ml for 231 cells and 1×10^7 cells/ml for 435 cells. MDA-MB-231 cells and derivatives (0.5×10^6 in 0.2 ml) were injected i.v. into the lateral tail vein of 3- to 4-week-old female athymic mice (Harlan Sprague-Dawley, Indianapolis, IN). Mice were killed 4 weeks post-injection and examined for the presence of metastases. Lungs were removed, rinsed in water, and fixed in Bouin's solution before quantification of surface metastases as described previously (7).

Similar procedures were used for the spontaneous metastasis assay using MDA-MB-435 cells, except that 1×10^6 cells (0.1 ml) were injected into exposed axillary mammary fat pads of anesthetized 5- to 6-week-old female athymic mice. When the mean tumor diameter reached 1.5–2.0 cm, tumors were surgically removed under ketamine:xylazine (80–85 mg/kg:14–16 mg/kg) anesthesia, and the wounds were closed with sterile stainless steel clips. Four weeks later, mice were sacrificed, and visible metastases were counted. Lung tissues were handled as above. Metastases were also observed in the ipsilateral and contralateral axillary lymph nodes of control mice. Occasional recurrences developed at the site of tumor removal, but the presence of hematogenous metastases did not necessarily correlate with presence of recurrent tumor.

Animals were maintained under the guidelines of the NIH and the Pennsylvania State University College of Medicine. All protocols were approved by the Institutional Animal Care and Use Committee. Food and water were provided *ad libitum*.

Statistical Analyses. The number of lung metastases was compared for $BRMS1$ transfectants and corresponding parental and vector-only-transfected MDA-MB-435 and MDA-MB-231 cells. For experimental metastasis assays, one-way ANOVA followed by Tukey's Honestly Significant Difference *post hoc* test was used. For spontaneous metastasis assays, a Kruskal-Wallis ANOVA of ranks procedure was used with Dunn's *post hoc* test. Statistical differences in adhesion and motility assays were done using Student's *t* test comparing $BRMS1$-transfected to vector-only-transfected cells. Calculations were performed using SigmaStat statistical analysis software (Jandel Scientific, San Rafael, CA). Statistical significance was designed as $P \leq 0.05$ using two-tailed tests.

RESULTS

DD-RTPCR was used to compare gene expression in metastasis-competent (435) *versus* metastasis-suppressed (neo11/435) variants. Initially, 64 cDNA fragments were detected as up-regulated in the neo11/435 hybrids. A representative result is shown in Fig. 1*a*. Eighteen bands amplified reproducibly in replicate RT-PCR reactions (data not shown). Of those, six fragments exhibited ≥5-fold higher mRNA expression in

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[6] R. S. Samant, M. T. Debies, and D. R. Welch, unpublished observations.

Fig. 1. Identification of differentially expressed genes in metastasis-suppressed neo11/435 hybrid cell clones using differential display. *a*, DD-RTPCR result in which *BRMS1* was identified. Replicate reactions were done in parallel to compare parental, metastatic MDA-MB-435 (*Lanes 1* and *2*) with mixtures (1:1:1) of metastasis-suppressed neo11/435 clones A3, B1, and D1 (*Lanes 3* and *4*). *Lanes 2* and *4* contain twice as much starting material as *Lanes 1* and *3*. An equal mixture of neo11/435 hybrid cell clones was used to minimize the impact of tumor heterogeneity on the differential display reaction. *b*, Northern blot analyses using candidate differential display products as probes. Only candidates displaying ≥5-fold higher expression in neo11/435 hybrid cell clones are presented. Poly(A)+ mRNA (2 μg) was electrophoresed on denatured agarose gels, transferred to a nylon membrane, fixed, and probed with random prime-radiolabeled PCR products from the differential display reaction. Equal loading was verified by probing with *GAPDH* cDNA (data not shown). Approximate transcript sizes are depicted to the *right* of each gel. *c*, differential expression of the novel genes was quantified using phosphorimage analysis. Relative expression was compared with parental MDA-MB-435, and only genes showing ≥5-fold higher (reference line shown as *dotted line*) expression in the neo11/435 hybrid cell clones were chosen for further study. *d*, multi-tissue Northern blots (Clonetech) showing mRNA expression of *BRMS1*.
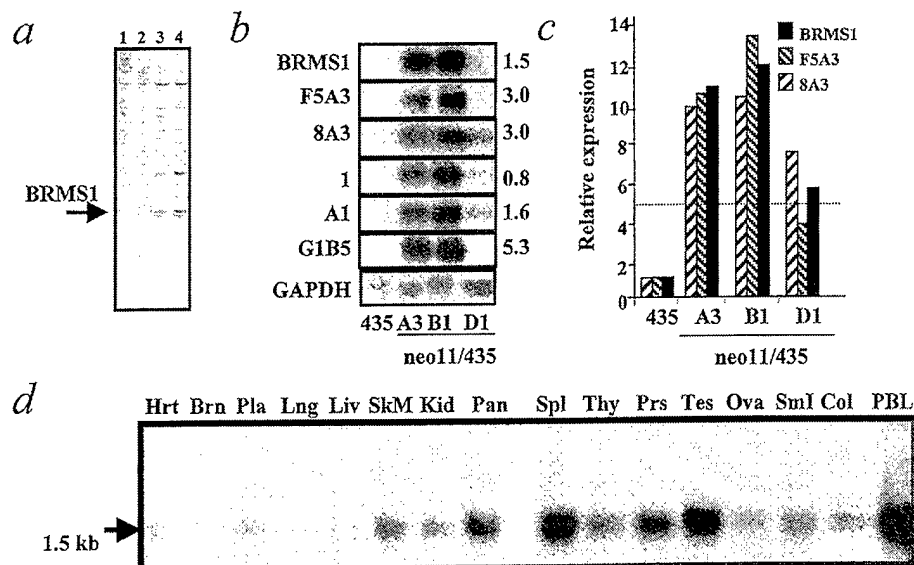
neo11/435 hybrid cell clones as detected by Northern blotting using the PCR product as a probe and quantified by phosphor image analysis (Fig. 1, *b* and *c*). None of the PCR products detected mRNAs expressed exclusively in the metastasis-suppressed cells. The differentially expressed cDNA inserts were sequenced, and homology to known genes and ESTs was assessed by comparing with the GenBank/European Molecular Biology Laboratory/DDBJ/PDB combined database. Three of the cDNAs were homologous to known human genes (*N*-acetylgalactosamine-6-sulphatase, adenine phosphoribosyltransferase, and hexokinase II). The remaining three cDNA fragments were novel and became priorities for further study. Here we report on the isolation and functional characterization of one of these novel cDNAs, *BRMS1*.

Full-length clones were obtained from a human kidney cDNA λTripIEx library after this tissue was found to express high levels of all three novel cDNAs. Tissue-specific splice variants were not expected because only a single band of ~1.5 kb was detected. This pattern was replicated using full-length *BRMS1* cDNA as a probe. *BRMS1* is widely expressed to varying levels in every normal human tissue examined (Fig. 1*d*).

Nucleotide sequence analysis of *BRMS1* cDNA initially revealed no significant homologies to any known genes, ESTs, or proteins deposited in the databases. Regions of *BRMS1* cDNA showed numerous homologies to short ESTs isolated from fetal liver and spleen as well as the human tumor cell line HeLa, consistent with the wide tissue expression observed in the multitissue Northern blots. *BRMS1* has been detected in Southern blots in multiple species at the DNA level, including yeast, human, mouse, rat, rabbit, and cow (data not shown). A murine cDNA recently has been isolated and is 89.8% homologous at the nucleotide level,[7] suggesting that the gene is relatively well conserved. The *BRMS1* human cDNA sequence was submitted to GenBank as a novel human gene with an Accession Number of AF159141.

After the *BRMS1* sequence was submitted, a deposited partial sequence (Accession Number AL050008; designated "hypothetical human protein sequenced by AGOWA within the cDNA sequencing consortium of the German Genome Project") had 91% homology at the predicted protein level for the region bounded by amino acids 60–244. However, no information regarding function for AL050008 is available. This may indicate that there is a *BRMS1* gene family.

Computerized analysis (DNAsis; Hitachi Software) shows that the

BRMS1 cDNA length is 1485 bp with the largest open reading frame of 741 bp (from nucleotides 122 to 862; Fig. 2). *BRMS1* encodes a novel protein of 246 amino acids ($M_r \approx 28,500$), a result confirmed using *in vitro* transcription and translation (data not shown). The genomic structure of *BRMS1* is organized as 10 exons spanning ~10 kb.[8] Exon 1 is untranslated. Fluorescence *in situ* hybridization mapping places the location of *BRMS1* gene at human chromosome 11q13.1-q13.2 (Fig. 3*a*).

The predicted amino acid sequence of BRMS1 was analyzed for structural and sequence homologies to obtain clues regarding mechanism of action. The identified structural domains of the BRMS1 protein are shown schematically in Fig. 3*b*. Regions of homology were identified using the algorithms listed below. Using PROSITE[9] several putative phosphorylation sites for cAMP/cGMP ([R/K]₂-x-[S/T]; amino acids 55–58 and 240–243), protein kinase C ([S/T]-[R/K]; amino acids 111–113, 147–149, 190–192, and 200–202), and casein kinase II ([S/T]-x₂-[D/E]; amino acids 19–22, 30–33, 37–40, 39–42, 41–44, and 46–49) were detected. PSORT II[10] identified two putative nuclear localization sequences (amino acids 198–205 and 239–245), which were shown to be functional according to subcellular fractionation and immunofluorescence studies using BRMS1-transfected 231 cells (data not shown). For both studies, antibodies to epitope-tagged BRMS1 (SV40T901 on the NH₂ terminus) were used. Additionally, BRMS1 contains two coiled-coil (amino acids 51–81 and 147–180) motifs and several imperfect leucine zipper (L-x₆-L-x₆-L-x₆-L) motifs at amino acids 67–88, 131–152, 138–159, 153–174, and 160–181. No signal peptide motifs were identified, but there was a potential endoplasmic reticulum retention sequence at amino acids 243–246. These predictions were corroborated using the ExPASy search engine.[11] The four cysteine residues within the BRMS1 protein are apparently not utilized for intra- or interprotein disulfide linkages because mobility in SDS-PAGE is unaffected by reducing agents (data not shown).

To assess the effect of *BRMS1* on breast carcinoma biological behavior, *BRMS1* was transfected into two independently derived metastatic human breast carcinoma cell lines, MDA-MB-435 (435) and MDA-MB-231 (231). The morphologies of 435 and 231 *BRMS1*-transfected cells

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[7] R. S. Samant, M. T. Debies, and D. R. Welch, manuscript in preparation.

[8] R. S. Samant, M. T. Debies, M. J. Seraj, and D. R. Welch, manuscript in preparation.
[9] http://www.genebio.com/prosite.html.
[10] http://www.psort.nibb.ac.jp:8800.
[11] http://www.expasy.ch.

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agaaaagggagccgcgcagcgcctacgggagtccggcggcagcagccggtaccggcaaccacgggcagctctcagggaa
tctccgtcgtgaggccagaggctccagtccccgcgagtccag
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      ATG CCT GTC CAG CCT CCA AGC AAA GAC ACA GAA GAG ATG GAA GCA GAG GGT GAT TCT GCT
1-     M   P   V   Q   P   P   S   K   D   T   E   E   M   E   A   E   G   D   S   A   -20
      GCT GAG ATG AAT GGG GAG GAG GAA GAG AGT GAG GAG GAG CGG AGC GGC AGC CAG ACA GAG
21-    A   E   M   N   G   E   E   E   E   S   E   E   E   R   S   G   S   Q   T   E   -40
      TCA GAA GAG GAG AGC TCC GAG ATG GAT GAT GAG GAC TAT GAG CGA CGC CGC AGC GAG TGT
41-    S   E   E   E   S   S   E   M   D   D   E   D   Y   E   R   R   R   S   E   C   -60
      GTC AGT GAG ATG CTG GAC CTA GAG AAG CAG TTC TCG GAG CTA AAG GAG AAG TTG TTC AGG
61-    V   S   E   M   L   D   L   E   K   Q   F   S   E   L   K   E   K   L   F   R   -80
      GAA CGA CTG AGT CAG CTG CGG TTG CGG CTG GAG GAA GTG GGG GCT GAG AGA GCC CCT GAA
81-    E   R   L   S   Q   L   R   L   R   L   E   E   V   G   A   E   R   A   P   E   -100
      TAC ACG GAG CCC CTT GGG GGG CTG CAG CGG AGC CTC AAG ATT CGC ATT CAG GTG GCA GGG
101-   Y   T   E   P   L   G   G   L   Q   R   S   L   K   I   R   I   Q   V   A   G   -120
      ATC TAC AAG GGC TTC TGT CTG GAT GTG ATC AGG AAT AAG TAC GAA TGT GAG CTG CAG GGA
121-   I   Y   K   G   F   C   L   D   V   I   R   N   K   Y   E   C   E   L   Q   G   -140
      GCC AAA CAG CAC CTG GAG AGT GAG AAG CTG CTG CTC TAT GAC ACG CTG CAG GGG GAG CTG
141-   A   K   Q   H   L   E   S   E   K   L   L   L   Y   D   T   L   Q   G   E   L   -160
      CAG GAG CGG ATC CAG AGG CTG GAG GAG GAC CGC CAG AGC CTG GAC CTC AGC TCT GAA TGG
161-   Q   E   R   I   Q   R   L   E   E   D   R   Q   S   L   D   L   S   S   E   W   -180
      TGG GAC GAC AAA CTG CAC GCC AGA GGC AGC TCC AGG TCT TGG GAC TCC CTG CCG CCC AGC
181-   W   D   D   K   L   H   A   R   G   S   S   R   S   W   D   S   L   P   P   S   -200
      AAG AGG AAG AAG GCA CCT CTG GTT TCT GGC CCA TAC ATC GTG TAC ATG CTT CAA GAG ATC
201-   K   R   K   K   A   P   L   V   S   G   P   Y   I   V   Y   M   L   Q   E   I   -220
      GAC ATC CTG GAG GAC TGG ACA GCC ATC AAA AAG GCT AGG GCA GCT GTG TCC CCT CAG AAG
221-   D   I   L   E   D   W   T   A   I   K   K   A   R   A   A   V   S   P   Q   K   -240
      AGA AAA TCG GAT GGA CCT TGA ccctgctgttcacagccagggggaccctcagagcagctggcactgcaccca
241-   R   K   S   D   G   P   *
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ggattctcgtcttcctcctgcagacaggcggacccacaggcccctcagggtctgcccagccaggctcctgtggtgctgct
gggccctcccactccatctggcactggcctggactcctcctctgccctcctcgaggcctgcacagctgtggccgtggagc
tgacctgaccaggcaaggctgctgtctccatccctgagccgcctgccacctcccactcctgaagatccatctcttggggc
tcccctgacagagaagacagccgaagtcaaagccacatcctcttgctgatgttggatgcaggctgtccagcctcagggcc
agggagccaagtttccactgtgcgggaactctgagtcagaacgttgattatctgggggtcttgtccaccctggctggatc
tggaggcaagatgccaggcccccaaggtgttctcagggcaagttcttggtgtctgctttctcagattccaaggactgga
attaaaacctttcctgggaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa
aaaaaaaaaaaa
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Fig. 2. Nucleotide (*upper case letters*) and predicted amino acid sequence (in *bold*) of *BRMS1* cDNA. BRMS1 cDNA GenBank Accession Number is AF159141. *, termination codon.

were not noticeably or uniformly different from parental cells. Nor were *in vitro* growth rates or saturation densities different. In the experiment shown, *BRMS1*-transfected cells exhibited a slight delay following seeding; however, this did not impact routine culture. *BRMS1* transfectants also tended to aggregate more readily after detachment. However, these properties were not evident in every clone isolated. Surprisingly, 435 cells, but not 231 cells, transfected with *BRMS1* acquired an acute sensitivity to trypsin (data not shown). Whereas parental cells previously were routinely passaged using a mixture of trypsin and EDTA, the *BRMS1* transfectants died when exposed to even low concentrations of trypsin. Therefore, subsequent cultures were handled using EDTA to detach the cells from the substrata.

Clones representing low, medium, and high *BRMS1* mRNA and protein expression (the latter were evaluated using epitope-tagged BRMS1) were chosen for *in vivo* functional studies. The transfected cells were then tested for tumorigenicity and metastasis in athymic mice. *BRMS1*-transfected 231 cells were injected i.v. and assessed for their ability to form macroscopic metastases in lung. Compared with vector-only transfectants, *BRMS1* transfectants exhibited a dose-dependent, significant ($P < 0.001$) decrease in metastatic potential (Fig. 3c). Similar conclusions were drawn using mRNA to assess expression (data not shown). This implies that the epitope tag does not deleteriously influence BRMS1 functionality. As expected, the ability of 231 cells to form progressively growing tumors in the mammary fat pad was not suppressed. Because the parental 231 cell line does not metastasize from an orthotopic site, metastatic suppression using the spontaneous metastasis assay could not be assessed.

Parental and *BRMS1*-transfected MDA-MB-435 cells grew progres-

sively after injection into an orthotopic (*i.e.*, mammary fat pad) site. Growth of *BRMS1*-transfected 435 tumors was somewhat delayed compared with the parental and/or vector controls. In general, once the tumors began to grow, their sizes were ~1 week behind the parental, metastatic populations, suggesting that rate of tumor growth, once established, is the same. Failure to suppress tumorigenicity indicates that *BRMS1* is not a tumor suppressor gene. The histologic appearance of parental 435 and *BRMS1* transfectants were similar, except that the latter exhibited fewer fibrous bands in the stromal compartment of the tumors (data not shown). Whereas tumorigenicity was unaltered, the incidence and number of metastases to lung and regional lymph nodes were significantly ($P = 0.004$) suppressed in the MDA-MB-435 *BRMS1* transfectants (Table 1). Parental and vector-only transfectant cells formed axillary lymph node and lung metastases in 100% of the mice injected, whereas in the *BRMS1* transfectants, the incidence dropped by 50–90%. Of the metastases that formed, all were noticeably smaller than the parental lesions at a comparable time following injection. Even if the metastases were given more time to grow, most did not develop into grossly visible lesions. BRMS1 expression was still detectable in the *BRMS1*-transfected 435 locally growing tumors. Because parental 435 cells do not form lung metastases following i.v. injection, i.v. inoculation studies like those with 231 were not done.

DISCUSSION

DD-RTPCR was used to discover genes more highly expressed in metastasis-suppressed neo11/435 than in their metastatic counterparts. *BRMS1*, which maps to a region frequently involved in breast carci-
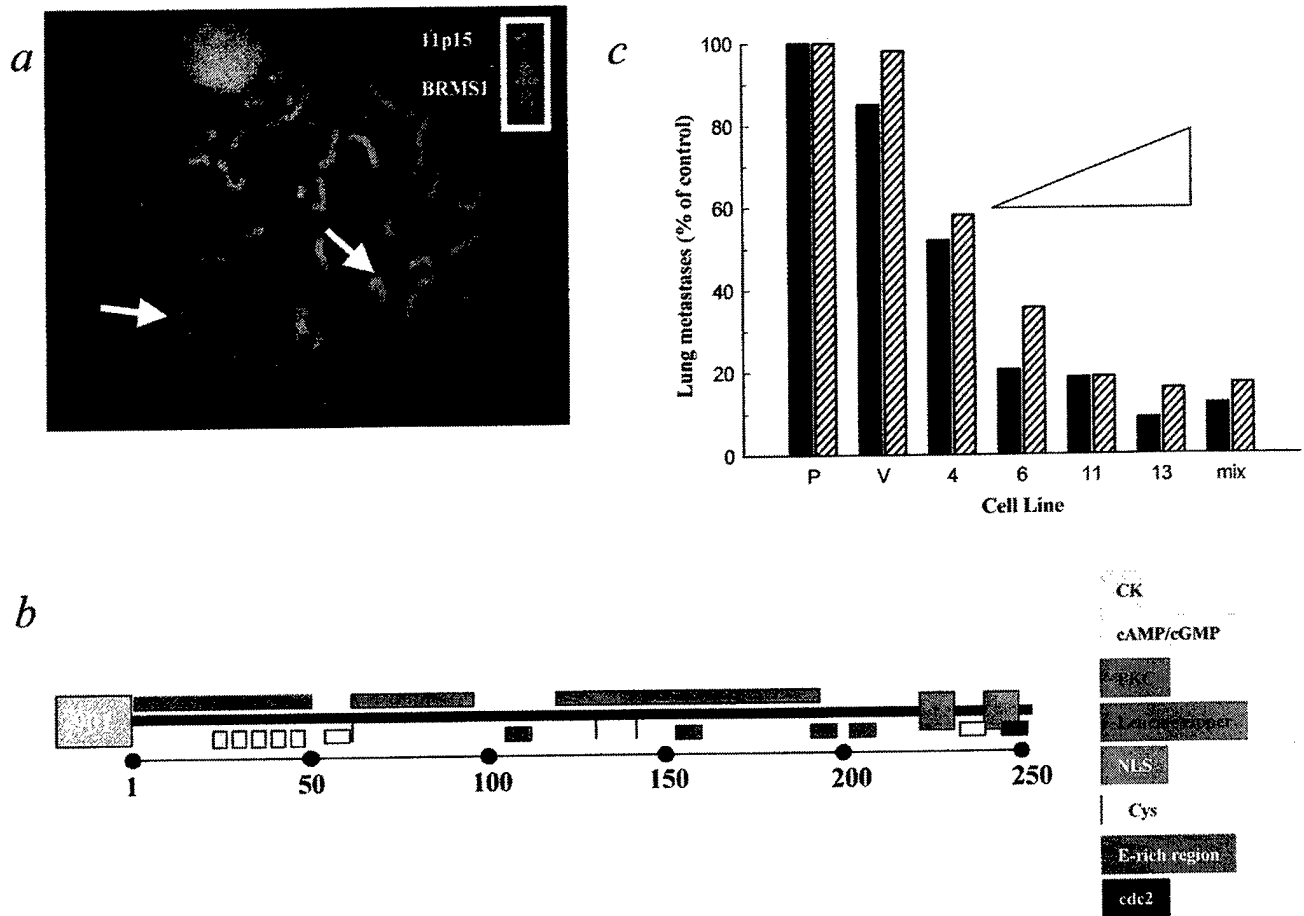
Fig. 3. *a*, The *BRMS1* gene maps to 11q13.1 by fluorescence *in situ* hybridization. *Arrows* show two chromosome 11 with *BRMS1* staining. An 11p15 probe and *BRMS1* probe are shown on DAPI-counterstained chromosome spread from normal human fibroblasts. *Inset* shows magnified chromosome 11 probed with two markers. *b*, schematic representation of the predicted domains of the BRMS1 protein. Phosphorylation domains for casein kinase (*CK*), *cAMP/cGMP*, and protein kinase C (*PKC*) are shown. Several imperfect *leucine zipper* motifs are located within the regions highlighted. A bipartite nuclear localization signal (*NLS*) is present at the COOH terminus, and a glutamate-rich region is located near the NH$_2$ terminus. *c*, BRMS1 suppresses metastasis of MDA-MB-231 cells. Parental line (P) and pcDNA3 vector-only transfectant (V) are controls for two independent experiments (*filled* and *hatched columns*). An uncloned population of BRMS1-transfected MDA-MB-231 (*mix*) is also included in these experiments. Metastasis suppression generally correlated with the level of BRMS1 mRNA and protein expression (triangle) as determined in independent experiments.

Table 1 *BRMS1 suppresses metastasis of MDA-MB-435*

Data are pooled from two independent experiments involving seven to eight mice per group. Data for the native BRMS1 (*i.e.*, not epitope-tagged) was collected from a single study.

		Incidence of metastases[b]			
		Lung		Extrapulmonary	
Cell line	Mean tumor diameter[a] (mm)	No. mice with metastases/No. mice injected	$P < 0.05$	No. mice with metastases/No. mice injected	$P < 0.05$
MDA-MB-435	7.8 ± 0.6	9/15		15/15	
MDA-MB-435/pcDNA3	8.9 ± 1.1	9/13		13/13	
BRMS1.3[901-epitope]	6.3 ± 0.3	2/15	***	3/15	***
BRMS1.4[901-epitope]	5.6 ± 0.4	5/14		5/14	
BRMS1.6[901-epitope]	5.8 ± 0.4	2/15	***	0/15	***
BRMS1.1	4.7 ± 0.3	2/8	***	0/8	***
BRMS1.3	4.7 ± 0.4	1/8		3/8	
BRMS1.5	6.7 ± 0.4	3/7		5/8	

[a] Cells (1 × 10^6) were injected into the axillary mammary pads of 5–6-week-old female athymic mice. To compare relative tumor growth, mean tumor diameter of tumors 42 days after injection is shown. To compensate for delayed tumor growth, tumors were removed when the mean diameter (square root of the product of orthogonal diameters) reached 1.3–1.5 cm. Four weeks later, mice were killed, and the presence of metastases determined.

[b] The number and incidence of lung metastases were compared with vector-only transfected MDA-MB-435 using the Kruskal-Wallis ANOVA followed by Dunn's post-test. Incidence of extrapulmonary metastases (usually ipsilateral axillary lymph nodes, but occasionally ribcage, diaphragm, and chest wall) was similarly examined.

noma progression (*i.e.*, 11q13.1-q13.2) was identified and was expressed 5–10-fold more in the metastasis-suppressed neo11/435 clones than in their metastatic parents. When transfected into MDA-MB-435 and MDA-MB-231 cells, both the incidence of metastasis and the number of lung metastases per mouse were significantly inhibited compared with controls. Although tumor development in BRMS1-transfected 435 cells was slightly delayed compared with controls, tumors still formed and grew at a similar rate. Even when tumor-bearing animals were allowed more time for metastases to grow (*i.e.*, to compensate for the slower growth of the locally growing

tumor), metastasis was suppressed. *BRMS1* mRNA was still detectable within the primary tumors (data not shown). Taken together, these data fulfill the functional definition that *BRMS1* is a metastasis-suppressor gene, *i.e.*, metastasis is suppressed, whereas tumorigenicity is not.

The mechanism by which BRMS1 suppresses metastasis is still not fully determined. *In vitro* assays assessing individual steps in the metastatic cascade predict a complex role for this molecule. The step(s) at which BRMS1 functions are downstream of local invasion: invasive cords are observed at the edge of locally growing tumors. This finding is consistent with the lack of gross changes in the ability of the cells to produce and activate matrix metalloproteinase-2 and -9 as detected by zymography.[6] Efficient invasion occurs despite a modest but reproducible decrease in motility as measured using *in vitro* wound migration assays. Adhesion to fibronectin, laminin, and type IV collagen are likewise unaffected by BRMS1 expression in 231 and 435 cells.[12]

Analysis of the predicted BRMS1 amino acid sequence hints that BRMS1 interacts with other proteins (*i.e.*, phosphorylation sites, coiled-coil periodicity, and leucine zipper). Because these motifs are often found in components of transcriptional machinery, we hypothesized that BRMS1 might suppress metastasis by regulating expression of other metastasis-suppressor genes; however, there appears to be no correlation between the expression of Kai1, Nm23, KiSS1 or E-cadherin with BRMS1.[6] Although this hypothesis is not formally disproved, these data argue that it is not the case.

BRMS1 transcript (1.5 kb) was detected in every human tissue examined, albeit at different levels. The uniform size of *BRMS1* transcript argues that it is not alternatively spliced in various tissues. Homology to *BRMS1* DNA was detected using Southern blotting. This suggests that *BRMS1* is relatively well conserved; however, the genomic organization is apparently different (data not shown).

Compared with the many normal tissues examined, *BRMS1* mRNA expression was very low in the 435 and 231 cells by both RT-PCR and poly(A)$^+$-enriched mRNA Northern blots. Analysis of protein levels awaits the development of BRMS1-specific antibodies (in progress). An RNA blot composed of a panel of human breast carcinoma cell lines with different malignant properties—MCF10A, MCF7, T47D^{CO}, MDA-MB-435, MDA-MB-231, LCC15, SUM185, SUM1315, and MKL-4 (8–12) was probed with full-length *BRMS1* cDNA to assess expression levels. These cell lines were chosen because all have characteristics that labeled them as "aggressive." In our hands, however, only 435 and 231 are reproducibly metastatic in athymic mouse models. *BRMS1* mRNA expression was high in LCC15 and MKL-4, but expression was also observed in MCF10A, T47D, SUM185, and SUM1315 cell lines.[6] Sequencing is underway to determine whether BRMS1 is wild-type or mutant in these cell lines.

In summary, we found a new human breast carcinoma metastasis suppressor gene by DD-RTPCR comparison of metastatic 435 cells

and metastasis-suppressed neo11/435 cells. The *BRMS1* gene maps to a "hot spot" in breast cancer progression, human chromosome 11q13, further supporting the likelihood that BRMS1 is important in human breast cancer progression toward metastasis. In general, low expression of BRMS1 correlates with the metastatic potential in human breast carcinoma cell lines in nude mice. It will be necessary to further analyze the *BRMS1* gene in other breast carcinomas during various stages of progression. Presently, it is not possible to state whether defects in human breast carcinoma are due to down-regulation of BRMS1, mutation, or both. Such experiments will require collection of matched samples from primary tumors and metastases. Because the most lethal attribute of breast cancer cells is their ability to spread and colonize distant sites, understanding BRMS1 function may help prevent metastasis and improve breast cancer survival.

REFERENCES

1. Welch, D. R., and Rinker-Schaeffer, C. W. What defines a useful marker of metastasis in human cancer? J. Natl. Cancer Inst., *91:* 1351–1353, 1999.
2. Welch, D. R., and Wei, L. L. Genetic and epigenetic regulation of human breast cancer progression and metastasis. Endocr. Relat. Cancer, *5:* 155–197, 1998.
3. Aldaz, C. M., Chen, A., Gollahon, L. S., Russo, J., and Zappler, K. Nonrandom abnormalities involving chromosome 1 and Harvey-*ras*-1 alleles in rat mammary tumor progression. Cancer Res., *52:* 4791–4798, 1992.
4. Phillips, K. K., Welch, D. R., Miele, M. E., Lee, J-H., Wei, L. L., and Weissman, B. E. Suppression of MDA-MB-435 breast carcinoma cell metastasis following the introduction of human chromosome 11. Cancer Res., *56:* 1222–1226, 1996.
5. Kierstead, T. D., and Tevethia, M. J. Association of p53 binding and immortalization of primary C57BL/6 mouse embryo fibroblasts by using simian virus 40 T-antigen mutants bearing internal overlapping deletion mutations. J. Virol., *67:* 1817–1829, 1993.
6. Liang, P., and Pardee, A. B. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science (Washington DC), *257:* 967–971, 1992.
7. Welch, D. R. Technical considerations for studying cancer metastasis *in vivo.* Clin. Exp. Metastasis, *15:* 272–306. 1997.
8. Soule, H. D., Maloney, T. M., Wolman, S. R., Peterson, W. D., Brenz, R., McGrath, C. M., Russo, J., Pauley, R. J., Jones, R. F., and Brooks, S. C. Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10. Cancer Res., *50:* 6075–6086, 1990.
9. Shafie, S. M., and Liotta, L. A. Formation of metastasis by human breast carcinoma cells (MCF7) in nude mice. Cancer Lett., *11:* 81–87, 1980.
10. Price, J. E., Polyzos, A., Zhang, R. D., and Daniels, L. M. Tumorigenicity and metastasis of human breast carcinoma cell lines in nude mice. Cancer Res., *50:* 717–721, 1990.
11. Forozan, F., Veldman, R., Ammerman, C. A., Parsa, N. Z., Kallioniemi, A., Kallioniemi, O. P., and Ethier, S. P. Molecular cytogenetic analysis of 11 new breast cancer cell lines. Br. J. Cancer, *81:* 1328–1334. 1999.
12. Kurebayashi, J., McLeskey, S. W., Johnson, M. D., Lippman, M. E., Dickson, R. B., and Kern, F. G. Quantitative demonstration of spontaneous metastasis by MCF7 human breast cancer cells cotransfected with fibroblast growth factor 4 and LacZ. Cancer Res., *53:* 2178–2187, 1993.

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[12] T. Sakamaki and D. R. Welch, unpublished observations.

Metastasis-Suppressor Genes: A Review and Perspective on an Emerging Field

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Figures: 3

Tables: 2

Abstract

1

Metastasis is the most lethal attribute of a cancer. There is a critical need for markers that will distinguish accurately those histologic lesions and disseminated cells, which have a high probability of causing clinically important metastatic disease, from those which will remain indolent. While the development of new diagnostic markers of metastasis was the initial motivation for many studies, the biological approach used to identify metastasis-suppressor genes has provided surprising insights into the *in vivo* mechanisms regulating the formation of metastases. This review and perspective describes the evolving view of the mechanisms that regulate metastasis and the importance of metastasis-suppressor genes in this process. The known metastasis-suppressor genes and the microcell-mediated chromosomal transfer strategy used to identify many of them are reviewed. New evidence for the role of these metastasis-suppressor activities (genes) in regulating the growth of disseminated cancer cells at the secondary site, the potential for the identification of novel therapeutic targets, and the multidisciplinary approach needed to translate this information into clinical tools for the treatment of metastatic disease are discussed.

The Clinical Problem: Predicting Metastatic Propensity

Our ability to detect and successfully treat localized cancers has improved appreciably in recent years. However, metastatic disease presents a continuing therapeutic challenge and is the predominant cause of death for most cancer patients. Thus, there is an emphasis on the diagnosis of cancers at an early stage when they are localized and most probably curable. Although screening for early stage disease is logical, its utility is limited by the inability of conventional diagnostic and histologic parameters to predict accurately the true extent and prognosis of a substantial proportion of clinically localized cancers (1-3). This is due, in part, to the inherent limitations and subjectivity of current grading and staging systems (4, 5).

The incidence of disease recurrence in surgical patients treated for prostatic and breast cancer illustrates this problem particularly well. Although we have a wealth of clinical and biological information, a large percentage of apparently resectable and theoretically curable lesions are found to be more advanced at the time of resection than envisaged, resulting in a substantial failure rate after attempted curative surgery (6-8). In prostate cancer patients, even when patient selection involves the exclusion of men with "high risk factors" (e.g. poorly differentiated histology, high PSA, clinical suspicion of local invasion) the relapse rate after radical retroperitoneal prostatectomy approaches 20-30% (9-11). Similarly, one-third of node negative breast cancer patients develop metastases, while the other two-thirds, despite receiving no chemotherapy, do not (12). Even in patients with small tumors and negative lymphnodes (T1N0), there is a 15 to 25 % likelihood of distant metastases (8).

Since the current staging system does not accurately identify those patients curable by regional treatment alone, the evaluation of additional parameters associated with the metastatic phenotype will be very important for the differentiation of patients curable by surgery alone from those requiring systemic therapy. For instance, men at high risk for relapse of prostate cancer can be identified (e.g. serum PSA > 10 ng/ml, clinical stage T_1 or T_2 with greater that 50% Gleason grade 4 on biopsy or clinical stage T_3 prostate cancer) and would be immediate candidates for adjuvant anti-metastatic therapies if they existed (10, 11, 13-16). Likewise, breast cancer patients with particularly poor prognoses can be identified by the detection of high microvessel counts

concurrent with low Nm23 and/or low E-cadherin expression in the primary tumor (12-17). In fact, these parameters are more significant prognostic biomarkers than the conventional analysis of tumor size and grade. The information obtained from the simultaneous evaluation of biomarkers such as these, will potentially lead to a reduction in the morbidity in those patients not requiring chemotherapy and possibly identify those patients requiring more aggressive therapies than currently used.

Overall, it is clear that there is a critical need for markers that will distinguish accurately those histologic lesions and disseminated cells, which have a high probability of causing clinically important metastatic disease, from those which will remain indolent (5, 15). Concerns have been raised that "metastasis" has often occurred by the time of diagnosis of the primary tumor, the implication being, that it is then too late for anti-metastatic therapy to be of use (18). However, the mere spread of cancer cells into the vasculature or to a secondary site does not constitute a metastatic lesion. Development of clinically significant metastases requires that a cancer cell complete a series of well-defined steps, generally referred to as the metastatic cascade (13). If a cell fails to complete any one of these steps, overt metastases will not develop (13-15). The clinical importance of disseminated cancer cells (detected by sensitive methods such as RT-PCR) has become an issue of considerable interest (19). Several such studies have reported the detection of tumor-derived cells in the circulation and bone marrow without future development of disease (16, 20, 21), while other reports have demonstrated an increased risk of disease recurrence in patients with bone marrow micrometastases both for prostate cancer, by the detection of mRNA transcripts for prostate specific antigen (22), and breast cancer, by the detection of cytokeratin-positive cells (23). Even in these later studies, however, the majority of patients with positive bone marrow samples did not actually develop recurrent disease, although the percentages may increase given extended time for patient follow-up. The discrepancy regarding the clinical importance of disseminated cells is likely due to differences in the experimental approaches used to identify cells (i.e. RT-PCR vs. immunohistochemical detection).

Tumor cell growth at the metastatic site is an important clinical target as cells must survive and proliferate in order to grow into overt, macroscopic metastases. The first step toward developing effective therapies to inhibit such growth is to identify the

genes/proteins that regulate metastatic colonization. To this end, a growing number of laboratories are focusing their translational research efforts on the discovery of genes that specifically regulate the metastatic ability of cancer cells. For example, several metastasis-promoting genes, including, *WDNM-1, WDNM-2, MMP11* (stromelysin-3), *MTA1,* and *ERBB2,* have been identified in association with the development of metastatic breast cancer (24-28). One must keep in mind, however, that it takes the coordinated expression of many genes to allow the development of metastases (29, 30). Thus, while it is relatively easy to demonstrate an association for a given gene with metastatic ability, it is difficult to prove that a particular gene is essential. On the other hand, in experimental systems it takes only one gene to block metastasis formation. Metastasis-suppressor genes suppress the formation of spontaneous, macroscopic metastases, *without affecting the growth rate of the primary tumor.* It has now been more than ten years since the discovery of the first metastasis-suppressor gene *nm23* (NME1) (31). Since then, reports have documented the important role of the loss of metastasis-suppressor gene function in the acquisition of metastatic ability (15, 31-33).

While the initial motivation for these studies was the development of new diagnostic markers of metastasis, the biological approach used to identify metastasis-suppressor genes has provided surprising insights into the *in vivo* mechanisms regulating the formation of metastases. We anticipate that identifying the molecular pathways that regulate metastatic colonization and growth control at the secondary site will provide additional, potentially novel therapeutic targets for the treatment of metastatic disease. The purpose of this review is to:

1. Present the evolving view of the mechanisms that regulate metastasis
2. Describe the functional strategy used to identify metastasis-suppressor genes and discuss important principles learned from these studies
3. Document the known metastasis-suppressor genes and report new evidence that supports their role in the regulation of growth control at the secondary site
4. Discuss the multidisciplinary approach needed to translate metastasis-suppressor genes into clinical tools

Regulation of Metastatic Propensity – Evolving Paradigms

Metastasis is defined as the formation of progressively growing secondary tumor foci at a site discontinuous from the primary lesion (15). This process is illustrated by the spontaneous hematogenous metastasis of tumor cells to the lung (Figure 1, Panel A). The formation of a primary tumor requires a cadre of molecular and cellular alterations that enable a cell(s) to circumvent normal growth control mechanisms, as well as, to manipulate its local environment (14). These changes include the development of a blood supply once the focus of transformed cells grows beyond a size that can be nourished by nutrient or metabolite diffusion (34, 35). Tumor progression and the acquisition of metastatic competence requires additional gene expression changes (e.g. protein degrading enzymes, adhesion molecules) that culminate in a malignant phenotype. Following invasion into adjacent tissues, tumor cells disseminate via blood vasculature or lymphatics and travel individually or as emboli comprised of tumor cells or tumor and host cells. At the secondary site, cells or emboli arrest either because of their physical size or by binding to specific molecules in particular organs or tissues (15, 36). In order for disseminated cells to grow into overt metastases, they must survive and proliferate in the vasculature or in the surrounding tissue after extravasation. The formation of clinically important metastases is dependent upon the completion of *every step of this cascade*, the last of which is metastatic colonization (Figure 1) (14). The presence of isolated cells at a secondary site represents a risk to the patient. Cells getting to the secondary site certainly have the potential to colonize, and therefore, it is crucial not to ignore the presence of neoplastic cells anywhere. On the other hand, as we will show, the mere presence of cells does not necessarily mean that metastatic colonization will occur. The challenge is to determine how to discriminate between disseminated cells that will form overt metastases from those that will not.

Cancer metastasis, both clinically and experimentally, is known to be inefficient (37). In experimental models, less than 0.1 % of cells injected into the circulation go on to form secondary tumors (15, 38). While many factors contribute to metastatic inefficiency, those considered to be most important include cell survival in, and escape from, the circulation (18). This process has, for the most part, been studied using endpoint assays in which the input (the number and kind of cells injected) is known and the endpoint (the numbers and sizes of metastases formed) is assessed (18, 39). The

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processes that control metastatic efficiency *in vivo* remain hidden, thus mechanistic paradigms have largely been based upon logical inference rather than direct observation. The development of new technologies have enabled researchers to test the possibility that cancer cell dissemination, arrest, and growth at the secondary site are critical determinants in metastasis formation.

The ability to observe single cells has been greatly enhanced by improvements in intravital microscopy and the utilization of vital fluorescent dyes like green fluorescent protein (GFP) (18, 40). Studies which couple these two powerful techniques have added greatly to our knowledge of the metastatic process following tumor cell entry into circulatory compartments. The use of *in vivo* video microscopy allows for the direct observation of experimental metastasis over time (39). Cancer cells can be fluorescently labelled *in vitro* and then injected into an animal. The cells can then be viewed at different time points, by both fluorescence and oblique transillumination, in thin tissues or superficial (≤ 50 µm) regions of thick tissues *in vivo* (39). Experiments utilizing this technology have demonstrated that, in contrast to the long-held belief, the vast majority of cancer cells in the microcirculation manage not only to survive there, but extravasate into the surrounding tissue within 1 to 2 days (41, 42). These studies have translated well into the clinical arena. Specifically, the vast majority of clinical studies using RT-PCR to detect prostate tumor cells in the peripheral circulation and bone marrow, found no correlation with disease failure (16, 20, 21). Taken together, the clinical and experimental evidence supports the observation that dissemination from the primary tumor site is a frequent event. Furthermore, these independent and complementary studies strongly suggest that growth control of individual disseminated cells determines the efficiency of metastatic colonization.

Metastatic Colonization

Metastatic colonization is the lodging and subsequent growth of disseminated cancer cells into clinically significant metastases (Figure 1, Panel B). In order to proliferate, surviving disseminated tumor cell(s) must be able to enact cell appropriate context-dependent signaling cascades, which enable them to survive, enter the cell cycle, and divide. While disseminated cells are likely to be present in numerous organs, only

certain environment(s) appear to be permissive for their survival and subsequent growth (38, 43, 44). Intercellular interactions with the stroma and with other tumor cells are critical for tumor cell survival and involve the activation of adhesion-dependent survival pathways such as those described for e-cadherin (45, 46) and integrin molecules (47). Clusters of proliferating cells grow into lesions consisting of a few hundred that can be detected histologically. Cells within such microscopic lesions can receive oxygen and nutrients by diffusion. Progressive growth of microscopic lesions into overt, or macroscopic metastases which are greater than 1 mm in diameter, requires that the fraction of proliferating cells exceed the fraction that are quiescent or apoptotic. In recent literature, this transition from microscopic metastasis to a macroscopic metastasis has often been referred to as the "switch to an angiogenic phenotype" or "the angiogenic switch" (48). The implication of this terminology is that microscopic metastases exist in one of two states; either the lesion is forming new blood vessels (angiogenic) or not. However, the progression from a "microscopic lesion" to an overt metastasis is more accurately described in terms of growth control. Indeed, the interchangeable use of "angiogenesis" and "growth" has been a source of confusion. This progression may occur over a period of months or even years and is not necessarily dependent upon new blood vessel formation. Vascularization is in fact a late step in metastatic colonization (49). Recent studies have shown that in addition to the induction of classical neovascularization via endothelial cell recruitment, tumor cell masses can develop a blood supply by alternative means such as the cooption of preexisting host vessels (49) or by formation of tumor channels, a process referred to as vascular mimicry (50). As will be described in the following paragraphs, recent data from our laboratories suggests that a subset of metastasis-suppressor genes inhibit earlier steps in metastatic colonization, prior to the need for development or recruitment of vessels.

Identification of Metastasis-Suppressor Activity: A Functional Approach

Metastasis-suppressor genes are genes that suppress the formation of (spontaneous) macroscopic metastases. As their name implies, these genes are distinct from *oncogenes*, which promote cellular transformation, and *tumor-suppressor genes*, which suppress tumor growth. While the first metastasis-suppressor gene, *nm23* was identified by a cDNA subtraction approach, the majority of metastasis-suppressor activities identified to date, have been discovered using microcell-mediated chromosomal transfer (MMCT; Table 1). The choice of MMCT strategy was logical as the existence of metastasis-suppressor genes was originally implicated by the results of somatic cell fusion studies; the precursor of MMCT (51-54). The techniques for the generation of genetically stable somatic cell hybrids were developed in the early studies of Barski, Ephrussi, Okada, and Harris (55). In most instances, fusion between malignant cells and normal cells results in hybrid cells which are suppressed in their tumorigenic capacity (56). Ichikawa *et al*, were the first to identify specific chromosomal losses associated with the reacquisition of metastatic ability (57). In their study, fusion of nonmetastatic and highly metastatic Dunning rat prostatic cancer cells resulted in nonmetastatic hybrids. Importantly, the tumorigenicity (e.g. tumor take and latency period) and *in vivo* growth rates of the primary tumors of hybrid clones containing a full complement of rat chromosomes were not affected. At the experimental endpoint, none of the animals bearing hybrid tumors developed distant metastases. However, when the nonmetastatic primary tumors were serially passaged *in vivo*, animals occasionally developed distant metastases. Cytogenetic analysis of these metastatic revertants revealed a consistent loss of a copy of rat chromosome 2. This critical study suggested that the loss of specific chromosomes could increase the metastatic potential of prostate cancer cells without affecting growth rate or tumorigenicity.

The observation of a metastasis-suppressor activity being associated with a specific chromosome occurred coincident with the development of MMCT as a technique for the study of genes encoded by individual human chromosomes (51-54, 58-61). In this approach, summarized in Figure 2, well-characterized donor cells, carrying a single human chromosome tagged with a selectable marker(s) (e.g. neomycin

phosphotransferase, etc.) are used to transfer the chromosome of interest into recipient cells (62). Briefly, donor cells are sequentially treated with colcemid, to depolymerize microtubules, and cytochalasin-B, to depolymerize actin bundles. The treated cells are centrifuged and the resulting pellet contains the microcells. Microcells are, in effect, micelles that contain single or multiple chromosomes. To enrich for those containing single chromosomes, the microcells are size-fractionated by sequential filtration through polycarbonate membranes of decreasing pore size. Microcells become attached to recipient cells in the presence of phytohemagglutinin and then become fused with the addition of polyethelene glycol. Recipient cells containing human chromosomes are selected in G418-containing media and then characterized by molecular and cytogenetic methods such as sequence tagged site PCR, karyotyping and fluorescence *in situ* hybridization (63, 64). The complete characterization of the hybrids under study is critical, as it provides information on the addition and /or deletion of donor and recipient chromosomal material, as well as, any rearrangements that may have occurred during MMCT.

Several laboratories have employed the technique of MMCT to test the functional significance of chromosomal alterations, such as LOH, observed in clinical samples. In addition, the use of MMCT has allowed the functional identification of genes conveying phenotypes such as senescence or tumor and metastasis-suppression (15, 65, 66). A review of the literature shows that transfer of a given chromosome can have different phenotypic effects that are dependent on the characteristics of the recipient cell line. For example, transfer of human chromosome 7 into an immortalized the SUSM-1 fibroblast cells induces senescence (67), while transfer of the same chromosome into a choriocarcinoma cells results in tumor suppression (68). Such results have enabled the definition of complementation groups for particular chromosome functions. The potential outcomes of transferring a particular chromosome into highly metastatic cells are summarized in Figure 2.

Studies using highly metastatic Dunning rat prostatic cells as the recipients for chromosomal transfer showed that chromosomes 12 and 17 specifically suppressed the metastatic ability of these cells (63, 64). The observed metastasis suppression had no effect on tumor growth rate. Interestingly, in analogous studies, transfer of these

chromosomes suppressed the tumorigenicity of human prostate cancer cell lines (69, 70). These findings could result from at least three alternative mechanisms. First, a given chromosomal region may encode a number of different genes, one or more of which may be active as a tumor-suppressor gene in human prostate cancer cells but inactive or not expressed in rat prostate cancer cells. Second, genes may function as metastasis-suppressor genes when expressed in rat prostate cancer cells but may be inactive or not expressed in human prostate cancer cell lines. Third, the gene(s) which lie on the same chromosomal region may have different functions depending on the context of the cell (i.e. cell type) in which they are expressed.

In this third case, the effect of the gene product may be "limited or determined" by the recipient cells. We refer to this scenario as our "cellular hard-wiring" hypothesis[1]. For example, human prostate cancer cell lines, as compared to Dunning rat prostatic cancer cell lines, are weakly metastatic in spontaneous metastasis assays (62). These differences in their *in vivo* biological activities could be the result of genetic differences between the tumor cells, or they could result from an epigenetic mechanism such as differential tumor-stromal interactions. The nature of cellular interactions with the extracellular matrix can regulate tissue-specific gene expression as cells form an elaborate three-dimensional network composed of the nuclear, cytoskeletal, and extracellular matrices (28, 71). Thus, the differential effects of a given chromosome transferred into different cell types can be the result of differential expression of the genes on the chromosome as determined by the way a cell responds to its environment.

During the past decade, several human chromosomes have been functionally tested through the use of MMCT, and metastasis-suppressor activities have been reported on chromosomes 1, 6, 7, 8, 10, 11, 12, 16 and 17 (63, 64, 72-82) (Table 1). Such functional studies have enabled the identification of KAI1, KISS-1, *MKK4*/MAP2K4 and BRMS1 as metastasis-suppressor genes.

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[1] In our work, the concept of cellular hardwiring refers to the work of Pienta and Coffey (71, 132).

Metastasis-Suppressor Genes

As discussed in the introduction, metastasis-suppressor genes suppress the formation of spontaneous, macroscopic metastases, *without affecting the growth rate of the primary tumor.* To date, five genes, *nm23*/NME1, KAI1, KISS1, BRMS1, and *MKK4*/MAP2K4, have been shown to meet the criteria of a metastasis-suppressor gene [Summarized in Table 2; (32, 33, 83-119)]. The role of other genes, such as CD44 and *maspin*/PI5, in metastasis suppression is less well-defined (102, 120-131). The potential mechanism of action of all of these genes has been inferred by analogy to other family members and observations in model systems. How these genes, and their protein products, function to suppress metastasis *in vivo* is the subject of enthusiastic study. Decreased expression of the suppressor gene is the key parameter determining metastatic potential and may occur by a variety of mechanisms not necessarily loss of heterozygosity (33, 91). At this time, *nm23*/NME1 and KAI1 are the most well-characterized metastasis-suppressor genes to date.

nm23/NME1

The prototypical metastasis-suppressor gene, *nm23*, was identified in the murine K1735 melanoma using subtractive hybridization; and six human homologs have been identified. Nm23-H1 expression has been correlated inversely with many, but not all, late-stage, metastatic human tumors (91). Transfection of *nm23*-H1 cDNA into highly metastatic murine melanoma, rat mammary adenocarcinoma, and human breast cancer and melanoma cells reduces their invasiveness and metastatic ability *in vivo* (91). In tumor cohorts, such as lung, colon, prostate, etc. [reviewed in reference (87)], where no alterations in the expression pattern of Nm23-H1 is evident, it is possible that the biological function of Nm23-H1 does not influence malignant progression in these cell types or that its effects are inhibited by alternate mechanisms. The mechanism of action for metastasis suppression by Nm23 still remains unknown, however, recent evidence suggests that it is phosphorylated and may be involved in a novel signaling pathway which, in turn, controls cell motility (84, 87).

KAI1

The localization of metastasis-suppressor activity to rat chromosome 2 in the cell fusion experiments by Ichikawa *et al.* prompted the search for homologous metastasis-suppressor genes for human prostate cancer. The first of such genes identified was *KAI1*. MMCT was used to transfer human chromosome 11 into Dunning AT6.1 and AT3.1 rat prostate cancer cells, and the resulting microcell hybrids were assayed for metastasis suppression in immunodeficient mice (81). These studies identified the metastasis-suppressor gene *KAI1*, which maps to 11p11.2-p13 (101). The metastasis-suppressor activity of *KAI1* was subsequently demonstrated by transfection into AT6.1 cells and assaying the metastatic ability of individual transfected control cell lines in severe combined immunodeficient (SCID) mice (101). Recent reports suggest that expression of KAI1 decreases both the invasiveness and motility of cells *in vitro* (101, 110). Additional studies show that *KAI1* transfectants exhibit enhanced Ca^{++}-independent aggregation indicating that KAI1 expression alters cell-cell interactions (109). In clinical studies, KAI1 protein expression was found to be down regulated in more than 70% of the primary prostatic cancers and in all of the lymph node metastases examined from untreated patients (33). Its down regulation has also been correlated with progression in a wide variety of cancers including pancreatic, hepatocellular, bladder, breast, and non small cell lung cancers (32, 133-136), as well as, esophageal cell carcinomas (137), squamous and lymphoid neoplasms (138). These data suggest that KAI1 has a conserved metastasis-suppressor function. Further, these studies demonstrate that metastasis-suppressor genes can be developed as clinical markers *even before their biochemical mechanism of action has been elucidated.*

Emerging Role of Metastatis-Suppressor Genes in the Regulation of Metastatic Growth

While it is tempting to speculate on the mechanism of action of genes listed in Table 2, examination of how genes such as *MKK4* or *BRMS1* suppress metastasis will require construction of appropriate biochemical constructs and identification of *in vitro* conditions that will enable us to conduct meaningful biochemical and molecular studies. As a first step to accomplishing this goal, our laboratories have initiated studies designed to examine the step in the metastatic cascade inhibited by a chromosome or gene of interest. As an example of these studies, we will present recent work on the metastasis-suppressor activity encoded by chromosomes 17 and 6. These studies have brought us closer to defining mechanisms of metastasis suppression.

Chromosome 17 Mediates Suppression of Growth at the Secondary Site

We have recently reported the identification of a discontinuous portion of human chromosome 17 (D17S952→D17S805, D17S930→ D17S797, and D17S944→ qter) that suppresses the metastatic ability of AT6.1 Dunning rat prostatic cancer cells when introduced via MMCT (63, 80). PCR and Southern blot analyses demonstrated that three of the four markers on 17p13, including *HIC1* and *TP53*, and 12 of the 13 markers in 17q21-23, including *BRCA1* and the metastasis-suppressor gene *NME1* (*nm23*), were not retained in this region (63). AT6.1 microcell hybrids containing this portion of chromosome 17 were tested in spontaneous metastasis assays. At the experimental endpoint, the number of overt surface metastases observed in the lungs from mice with AT6.1-17 tumors was reduced 15 to 30-fold as compared to lungs from mice bearing parental AT6.1 tumors (63). This suppression could be due to the inhibition of any step within the metastatic cascade. We reasoned that examination of the biology of metastasis suppression would provide clues to the identity of genes responsible for suppression of metastatic growth. A series of *in vivo* experiments were conducted and no evidence was found to suggest that there is a decrease in the number and/or viability of tumor cells colonizing the lung (80).

Based upon these findings, we hypothesized that a gene(s) encoded by the suppressor region of chromosome 17 functions by inhibiting the growth of metastases *in the lung* (139). To test this possibility, AT6.1-17 cells were transduced with a β-

galactosidase (β-gal) reporter gene construct (AT6.1-17Tβgal cells) and used in spontaneous metastasis assays (2). At the experimental endpoint, animals were sacrificed and the excised lungs were stained for β-gal expression. This approach allowed the visualization of microscopic AT6.1-17βgal surface metastases. Subcutaneous injection of AT6.1 parental cells results in the formation of a mean number of 97 overt surface metastases per lung (Fig. 3, A, *left*). As expected, the number of overt macroscopic metastases detected by Bouin's fixation after the subcutaneous (sc.) injection of AT6.1-17-Tβgal cells is greatly reduced. The use of Bouin's fixative allows for the visualization of macroscopic metastases; however, microscopic metastases are not detected (Fig. 3, A, *middle*). In contrast, when lungs removed from mice carrying AT6.1-17-Tβgal tumors were stained for β-gal activity, numerous blue-staining microscopic metastases were observed (Fig. 3, A, *right*). Interestingly, the mean number of AT6.1-17-Tβgal micrometastases (i.e. 62 ± 12) detected by this method is similar to the number of macroscopic AT6.1 metastases (i.e. 97 ± 6). These results demonstrate that AT6.1-17 cells do escape from the primary tumor and arrest in the lungs, but do not form large metastatic foci (139).

Because of the similarity between our findings to the angiostatin-mediated dormancy reported by Holmgren *et al.* (140), we investigated the possibility that AT6.1-17 primary tumors secrete a substance that suppresses the growth of its own metastases (139). For this experiment, 2 x 10^5 AT6.1-17 cells were injected subcutaneously into the flanks of SCID mice which were then divided into two experimental groups. Once the tumors reached a volume of 1 cm³, the tumors were surgically removed from the mice in the first group while those in the second group were left intact, although a contralateral sham surgery was performed. It was anticipated that if the AT6.1-17 primary tumor secretes a substance like angiostatin, which suppresses the growth of its own metastases, then a significant increase in the number of overt metastases should develop in the lungs of mice in which the primary tumors had been removed. However, after approximately 65 days post-injection, the animals were sacrificed and examination of the lungs from both groups showed no difference in the numbers of overt macrometastases (139). Thus, these studies found no evidence for an antiangiogenic mechanism in this model.

Taken together, our data suggested that AT6.1-17 cells escape from the primary tumor but are growth inhibited at the secondary site (139). If this is an early event, we predicted that viable, disseminated AT6.1 and AT6.1-17 cells should be present in the lung at very early time points. We found that viable cells could be harvested from the lungs of both AT6.1- and AT6.1-17- tumor bearers as early as 18 days post-injection (Figure 3, Panel B). Our preliminary time-course data show that AT6.1-17 cells disseminate and arrest in the lungs, but have an extended latency period as compared to AT6.1 parental cells.

Chromosome 6

Based upon the high incidence of chromosome 6 abnormalities in late-stage human melanoma, we introduced an intact chromosome 6 into the highly metastatic C8161 human melanoma cells by MMCT. Parental cells formed tumors in every mouse injected intradermally with 1×10^6 cells; and more than 90% of the mice developed regional lymph node and lung metastases. In contrast, chromosome 6-C8161 hybrids (neo6/C8161) were still tumorigenic but completely suppressed for metastasis from an orthotopic site (141). Intravenous injection of neo6/C8161 cells also did not produce metastases. Introduction of a chromosome 6 with deletions on the long arm recently mapped the metastasis-suppressor locus to a 40 Mb region represented by chromosomal bands 6q16.3-q23 (142).

The mechanism of action for the metastasis-suppressor was studied using a variety of *in vitro* and *in vivo* techniques. The neo6/C8161 cells were still locally invasive and cells were even detected in efferent vessels. This implied that the step(s) in the metastatic cascade inhibited by introduction of chromosome 6 were subsequent to intravasation. The identity of those steps was not further elucidated using *in vitro* assays mimicking adhesion, invasion, motility or growth. No significant differences between the metastatic and nonmetastatic cells were observed using the many *in vitro* assays (72, 73, 141, 143).

To better define the step(s) in metastasis blocked by addition of chromosome 6, cells that constitutively express green fluorescent protein (GFP) were engineered. GFP-tagged C8161 and neo6/C8161 cells were injected intravenously into athymic mice. C8161, as expected, formed overt metastases, but neo6/C8161 cells did not. Microscopic metastases (single cells or clusters of <10 cells) were observed in the lungs following

neo6/C8161 cell injection, suggesting that these cells lodged in the lungs but failed to proliferate (144). To determine whether the fluorescing cells were viable, they were isolated from lung up to 60 days post-injection and grown in culture. Upon injection into the skin of athymic mice, the neo6/C8161 cells grew at rates similar to previously uninjected neo6/C8161 cells. This result implies that the gene(s) on chromosome 6 interfere specifically with growth regulatory responses in the lung, but not in the skin.

From Gene Discovery to Clinical Utility

This review has focused on the identification and development of metastasis-suppressor genes as new additions to our molecular armamentarium. As translational researchers, our immediate goal is to: (1) improve the ability of the pathologist to unambiguously distinguish malignant from indolent lesions and (2) help the clinician differentiate tumors with a high likelihood of metastasis from those that do not. The practical question therefore, is how can we use these genes, or the pathways that they regulate, to improve patient management? When the search for metastasis-suppressor genes was initiated in the late 1980s, the major challenge was the identification of candidate genes. Recently, however, there has been an explosion in the genetic information that is instantly available. Furthermore, due to the efforts of independent laboratories and cooperative efforts, such as the Cancer Genome Anatomy Project of the NCI, cancer transcriptomes and proteomes will soon be available (145, 146). New technologies will continue to increase our ability to dissect molecular pathways in individual cells within human cancers. While this wealth of information will no doubt be of use, work from Bissell, Cuhna, and Chung has clearly demonstrated that tissue structure determines, or at least greatly influences, gene expression and function (147-154). Thus, it may be extremely difficult to predict the importance of genes expressed in individual microdissected cancer cells to the biology of the intact tumor, the behavior of which is determined by complex interactions of a population of cells. The present challenge is to identify the genes that are *functionally important* in the acquisition of metastatic ability (a real Y2K dilemma). Achieving this goal will require the use of well-characterized, *in vivo* (animal) models coupled with clinical correlative studies. It must be emphasized that *in vitro models do not accurately* reflect *in vivo* metastasis (155).

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Indeed, none of the metastasis-suppressor genes described herein could have been identified using traditional in vitro *assays.* Given the inherent variability and nonlinear behaviors of biological systems, it is probable that no one model will prove adequate in sorting out the contributions of the multiplicity of genes involved in the development of metastases. Thus, it is more advantageous to focus studies on a particular model and tease out important cellular pathways modulated by a particular gene of interest, and then, test and verify the importance of the target pathway in clinical disease, as well as, in additional model systems.

Technological advances are enabling us to examine the metastatic process and the genes that regulate it in new ways. This ability has caused us to reevaluate fundamental concepts concerning the determinants of metastatic propensity. In the past, the escape of cells from the primary site was viewed as the rate-limiting step for the development of metastases. The clinical implication being, that disseminated cancer cells were destined to become clinically important metastases, thus, "antimetastatic" therapies were no longer of use (18). Findings from clinical studies and basic research from several independent laboratories, have shown that survival and subsequent growth of extravasated cancer cells at the secondary site may determine metastatic efficacy. These observations are driving our laboratories, and others, to reconsider the role of endothelial cell- tumor cell interactions in survival signaling and growth control cascades in order to develop new strategies for controlling the growth of disseminated cancer cells (39, 45, 156).

As metastasis researchers, we find ourselves in the midst of a revolution. In preparing this review we considered the parallels between recent developments in our field and the development of the field of molecular biology. Much of early molecular biology was pursued by individuals who were not trained as biologists, but as physicists such as Max Delbrück (157). We are respectful of the observations of Erwin Schrödinger, the father of statistical mechanics, who observed that, "all of the physical and chemical laws that are known to play an important part in the life of organisms are of the statistical kind. The behavior of such systems depends entirely on a large number of molecules that cooperate to form the observed function or phenotype (158)." Although this comment was made in regard to normal biological processes, it is equally applicable

to the multiple genetic changes that are required for the acquisition of metastatic ability. Metastasis is a complex, multigenic phenotype. As such, multiple markers will be needed for the accurate assessment of metastatic ability. This is highlighted by the tremendous impact of seemingly trivial experimental manipulations on the outcome of metastasis assays (155). Recently, parallels have been drawn between the behavior of cancer cells and complex adaptive systems (159, 160). As such, very small changes in initial conditions may produce an outcome of such great diversity it seems random (159). Ultimately, we believe that in order to translate our molecular findings into meaningful markers, we will have to go beyond our traditional areas of expertise and work with mathematicians, computational biologists and others to take this revolution from bench to bedside.

References:

1.   Mackillop, W. J., O'Sullivan, B., and Gospodarowicz, M. The role of cancer staging in evidence-based medicine. Cancer Prev Control, 2: 269-277, 1998.

2.   Gospodarowicz, M., Benedet, L., Hutter, R. V., Fleming, I., Henson, D. E., and Sobin, L. H. History and international developments in cancer staging. Cancer Prev Control, 2: 262-268, 1998.

3.   Bostwick, D. G. Staging prostate cancer--1997: current methods and limitations. Eur Urol, 32: 2-14, 1997.

4.   Carter, H., Partin, A. W., Diagnosis of prostate cancer, in Campbell's Urology, R.A. Walsh, PC, Vaughn ED, Wein AJ, Editors. 1998 W.B. Saunders Company: Philaelphia, p 2519-2538.

5.   Isaacs, J. T. Molecular markers for prostate cancer metastasis. Developing diagnostic methods for predicting the aggressiveness of prostate cancer. Am J Pathol, 150: 1511-1521, 1997.

6. Schellhammer, P. F. Radical prostatectomy. Patterns of local failure and survival in 67 patients. Urology, *31*: 191-197, 1988.

7. Lerner, S. P., Seale-Hawkins, C., Carlton, C. E., Jr., and Scardino, P. T. The risk of dying of prostate cancer in patients with clinically localized disease. J Urol, *146*: 1040-1045, 1991.

8. Heimann, R., and Hellman, S. Clinical progression of breast cancer malignant behavior: what to expect and when to expect it. J Clin Oncol, *18*: 591-599, 2000.

9. Catalona, W. J., and Dresner, S. M. Nerve-sparing radical prostatectomy: extraprostatic tumor extension and preservation of erectile function. J Urol, *134*: 1149-1151, 1985.

10. Lu-Yao, G. L., McLerran, D., Wasson, J., and Wennberg, J. E. An assessment of radical prostatectomy. Time trends, geographic variation, and outcomes. The Prostate Patient Outcomes Research Team [see comments]. Jama, *269*: 2633-2636, 1993.

11. Voges, G. E., McNeal, J. E., Redwine, E. A., Freiha, F. S., and Stamey, T. A. Morphologic analysis of surgical margins with positive findings in prostatectomy for adenocarcinoma of the prostate. Cancer, *69*: 520-526, 1992.

12. Heimann, R., Lan, F., McBride, R., and Hellman, S. Separating favorable from unfavorable prognostic markers in breast cancer: the role of E-cadherin. Cancer Res, *60*: 298-304, 2000.

13. Poste, G., and Fidler, I. J. The pathogenesis of cancer metastasis. Nature, *283*: 139-146, 1980.

14. MacDonald, N. J., and Steeg, P. S. Molecular basis of tumour metastasis. Cancer Surv, *16*: 175-199, 1993.

15. Welch, D. R., and Rinker-Schaeffer, C. W. What defines a useful marker of metastasis in human cancer? J Natl Cancer Inst, *91*: 1351-1353, 1999.

16. Melchior, S. W., Corey, E., Ellis, W. J., Ross, A. A., Layton, T. J., Oswin, M. M., Lange, P. H., and Vessella, R. L. Early tumor cell dissemination in patients with clinically localized carcinoma of the prostate. Clin Cancer Res, *3*: 249-256, 1997.

17. Heimann, R., Ferguson, D. J., and Hellman, S. The relationship between nm23, angiogenesis, and the metastatic proclivity of node-negative breast cancer. Cancer

Res, *58*: 2766-2771, 1998.

18. Chambers, A. F. The metastatic process: basic research and clinical implications. Oncol Res, *11*: 161-168, 1999.

19. Gomella, L. G., Raj, G. V., and Moreno, J. G. Reverse transcriptase polymerase chain reaction for prostate specific antigen in the management of prostate cancer [see comments]. J Urol, *158*: 326-337, 1997.

20. Ellis, W. J., Vessella, R. L., Corey, E., Arfman, E. W., Oswin, M. M., Melchior, S., and Lange, P. H. The value of a reverse transcriptase polymerase chain reaction assay in preoperative staging and followup of patients with prostate cancer [see comments]. J Urol, *159*: 1134-1138, 1998.

21. Oefelein, M. G., Ignatoff, J. M., Clemens, J. Q., Watkin, W., and Kaul, K. L. Clinical and molecular followup after radical retropubic prostatectomy. J Urol, *162*: 307-310; discussion 310-301, 1999.

22. Wood, D. P., Jr., and Banerjee, M. Presence of circulating prostate cells in the bone marrow of patients undergoing radical prostatectomy is predictive of disease-free survival. J Clin Oncol, *15*: 3451-3457, 1997.

23. Braun, S., Pantel, K., Muller, P., Janni, W., Hepp, F., Kentenich, C. R., Gastroph, S., Wischnik, A., Dimpfl, T., Kindermann, G., Riethmuller, G., and Schlimok, G. Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer [see comments]. N Engl J Med, *342*: 525-533, 2000.

24. Ebralidze, A., Tulchinsky, E., Grigorian, M., Afanasyeva, A., Senin, V., Revazova, E., and Lukanidin, E. Isolation and characterization of a gene specifically expressed in different metastatic cells and whose deduced gene product has a high degree of homology to a Ca2+-binding protein family. Genes Dev, *3*: 1086-1093, 1989.

25. Dear, T. N., Ramshaw, I. A., and Kefford, R. F. Differential expression of a novel gene, WDNM1, in nonmetastatic rat mammary adenocarcinoma cells. Cancer Res, *48*: 5203-5209, 1988.

26. Dear, T. N., McDonald, D. A., and Kefford, R. F. Transcriptional down-regulation of a rat gene, WDNM2, in metastatic DMBA-8 cells [see comments]. Cancer Res, *49*: 5323-5328, 1989.

27. Basset, P., Bellocq, J. P., Wolf, C., Stoll, I., Hutin, P., Limacher, J. M., Podhajcer, O. L., Chenard, M. P., Rio, M. C., and Chambon, P. A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature, *348*: 699-704, 1990.

28. Fujita, M., Spray, D. C., Choi, H., Saez, J., Jefferson, D. M., Hertzberg, E., Rosenberg, L. C., and Reid, L. M. Extracellular matrix regulation of cell-cell communication and tissue-specific gene expression in primary liver cultures. Prog Clin Biol Res, *226*: 333-360, 1986.

29. Fidler, I. J., and Radinsky, R. Search for genes that suppress cancer metastasis [editorial; comment]. J Natl Cancer Inst, *88*: 1700-1703, 1996.

30. Fidler, I. J., and Radinsky, R. Genetic control of cancer metastasis [editorial]. J Natl Cancer Inst, *82*: 166-168, 1990.

31. Steeg, P. S., Bevilacqua, G., Kopper, L., Thorgeirsson, U. P., Talmadge, J. E., Liotta, L. A., and Sobel, M. E. Evidence for a novel gene associated with low tumor metastatic potential. J Natl Cancer Inst, *80*: 200-204, 1988.

32. Yang, X., Welch, D. R., Phillips, K. K., Weissman, B. E., and Wei, L. L. KAI1, a putative marker for metastatic potential in human breast cancer. Cancer Lett, *119*: 149-155, 1997.

33. Dong, J. T., Suzuki, H., Pin, S. S., Bova, G. S., Schalken, J. A., Isaacs, W. B., Barrett, J. C., and Isaacs, J. T. Down-regulation of the KAI1 metastasis suppressor gene during the progression of human prostatic cancer infrequently involves gene mutation or allelic loss. Cancer Res, *56*: 4387-4390, 1996.

34. Folkman, J. Angiogenesis and angiogenesis inhibition: an overview. Exs, *79*: 1-8, 1997.

35. Folkman, J. The role of angiogenesis in tumor growth. Semin Cancer Biol, *3*: 65-71, 1992.

36. Pasqualini, R., and Ruoslahti, E. Organ targeting in vivo using phage display peptide libraries. Nature, *380*: 364-366, 1996.

37. Fidler, I. J. Review: biologic heterogeneity of cancer metastases. Breast Cancer Res Treat, *9*: 17-26, 1987.

38. Fidler, I. J. Critical factors in the biology of human cancer metastasis: twenty-

eighth G.H.A. Clowes memorial award lecture. Cancer Res, *50*: 6130-6138, 1990.

39. Chambers, A. F., MacDonald, I. C., Schmidt, E. E., Vincent, L. M., and Groom, A. C. Clinical targets for anti-metastasis therapy. Adv Cancer Res, *79*:

40. Yang, M., Jiang, P., Sun, F. X., Hasegawa, S., Baranov, E., Chishima, T., Shimada, H., Moossa, A. R., and Hoffman, R. M. A fluorescent orthotopic bone metastasis model of human prostate cancer. Cancer Res, *59*: 781-786, 1999.

41. Chambers, A. F., MacDonald, I. C., Schmidt, E. E., Koop, S., Morris, V. L., Khokha, R., and Groom, A. C. Steps in tumor metastasis: new concepts from intravital videomicroscopy. Cancer Metastasis Rev, *14*: 279-301, 1995.

42. MacDonald, I. C., Schmidt, E. E., Morris, V. L., Groom, A. C., and Chambers, A. F. In vivo videomicroscopy of experimental hematogenous metastasis: cancer cell arrest, extravasation and migration in Motion Analysis of Living Cells. D. R. Soll and D. Wessels, Editors, John Wiley and Sons, New York, pp 263-287, 2000.

43. Radinsky, R., and Fidler, I. J. Regulation of tumor cell growth at organ-specific metastases. In Vivo, *6*: 325-331, 1992.

44. Fidler, I. J. Critical determinants of melanoma metastasis. J Investig Dermatol Symp Proc, *1*: 203-208, 1996.

45. Day, M. L., Zhao, X., Vallorosi, C. J., Putzi, M., Powell, C. T., Lin, C., and Day, K. C. E-cadherin mediates aggregation-dependent survival of prostate and mammary epithelial cells through the retinoblastoma cell cycle control pathway. J Biol Chem, *274*: 9656-9664, 1999.

46. Guilford, P. E-cadherin downregulation in cancer: fuel on the fire? Mol Med Today, *5*: 172-177, 1999.

47. Giancotti, F. G., and Ruoslahti, E. Integrin signaling. Science, *285*: 1028-1032, 1999.

48. Arbiser, J. L., Moses, M. A., Fernandez, C. A., Ghiso, N., Cao, Y., Klauber, N., Frank, D., Brownlee, M., Flynn, E., Parangi, S., Byers, H. R., and Folkman, J. Oncogenic H-ras stimulates tumor angiogenesis by two distinct pathways. Proc Natl Acad Sci U S A, *94*: 861-866, 1997.

49. Holash, J., Maisonpierre, P. C., Compton, D., Boland, P., Alexander, C. R., Zagzag, D., Yancopoulos, G. D., and Wiegand, S. J. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science, *284*: 1994-

1998, 1999.

50. Maniotis, A. J., Folberg, R., Hess, A., Seftor, E. A., Gardner, L. M., Pe'er, J., Trent, J. M., Meltzer, P. S., and Hendrix, M. J. Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry [see comments]. Am J Pathol, *155*: 739-752, 1999.

51. Turpeenniemi-Hujanen, T., Thorgeirsson, U. P., Hart, I. R., Grant, S. S., and Liotta, L. A. Expression of collagenase IV (basement membrane collagenase) activity in murine tumor cell hybrids that differ in metastatic potential. J Natl Cancer Inst, *75*: 99-103, 1985.

52. Sidebottom, E., and Clark, S. R. Cell fusion segregates progressive growth from metastasis. Br J Cancer, *47*: 399-405, 1983.

53. Ramshaw, I. A., Carlsen, S., Wang, H. C., and Badenoch-Jones, P. The use of cell fusion to analyse factors involved in tumour cell metastasis. Int J Cancer, *32*: 471-478, 1983.

54. Layton, M. G., and Franks, L. M. Selective suppression of metastasis but not tumorigenicity of a mouse lung carcinoma by cell hybridization. Int J Cancer, *37*: 723-730, 1986.

55. Sager, R. Genetic suppression of tumor formation. Adv Cancer Res, *44*: 43-68, 1985.

56. Harris, H. The analysis of malignancy by cell fusion: the position in 1988. Cancer Res, *48*: 3302-3306, 1988.

57. Ichikawa, T., Ichikawa, Y., and Isaacs, J. T. Genetic factors and metastatic potential of prostatic cancer. Cancer Surv, *11*: 35-42, 1991.

58. Fournier, R. E., and Ruddle, F. H. Microcell-mediated transfer of murine chromosomes into mouse, Chinese hamster, and human somatic cells. Proc Natl Acad Sci U S A, *74*: 319-323, 1977.

59. Ege, T., Ringertz, N. R., Hamberg, H., and Sidebottom, E. Preparation of microcells. Methods Cell Biol, *15*: 339-357, 1977.

60. Oshimura, M. Lessons learned from studies on tumor suppression by microcell-mediated chromosome transfer. Environ. health Perspectives *93*: 57-8, 1991.

61. Saxon, P. J., Srivatsan, E. S., Leipzig, G. V., Sameshima, J. H., and Stanbridge, E.

J. Selective transfer of individual human chromosomes to recipient cells. Mol Cell Biol, *5*: 140-146, 1985.

62. Rinker-Schaeffer, C. W., Partin, A. W., Isaacs, W. B., Coffey, D. S., and Isaacs, J. T. Molecular and cellular changes associated with the acquisition of metastatic ability by prostatic cancer cells. Prostate, *25*: 249-265, 1994.

63. Chekmareva, M. A., Hollowell, C. M., Smith, R. C., Davis, E. M., LeBeau, M. M., and Rinker-Schaeffer, C. W. Localization of prostate cancer metastasis-suppressor activity on human chromosome 17. Prostate, *33*: 271-280, 1997.

64. Luu, H. H., Zagaja, G. P., Dubauskas, Z., Chen, S. L., Smith, R. C., Watabe, K., Ichikawa, Y., Ichikawa, T., Davis, E. M., Le Beau, M. M., and Rinker-Schaeffer, C. W. Identification of a novel metastasis-suppressor region on human chromosome 12. Cancer Res, *58*: 3561-3565, 1998.

65. Chang, Z. F. Regulatory mechanisms of replication growth limits in cellular senescence. J Formos Med Assoc, *96*: 784-791, 1997.

66. Vojta, P. J., Futreal, P. A., Annab, L. A., Kato, H., Pereira-Smith, O. M., and Barrett, J. C. Evidence for two senescence loci on human chromosome 1. Genes Chromosomes Cancer, *16*: 55-63, 1996.

67. Nakabayashi, K., Ogata, T., Fujii, M., Tahara, H., Ide, T., Wadhwa, R., Kaul, S. C., Mitsui, Y., and Ayusawa, D. Decrease in amplified telomeric sequences and induction of senescence markers by introduction of human chromosome 7 or its segments in SUSM-1. Exp Cell Res, *235*: 345-353, 1997.

68. Matsuda, T., Sasaki, M., Kato, H., Yamada, H., Cohen, M., Barrett, J. C., Oshimura, M., and Wake, N. Human chromosome 7 carries a putative tumor suppressor gene(s) involved in choriocarcinoma. Oncogene, *15*: 2773-2781, 1997.

69. Murakami, Y. S., Brothman, A. R., Leach, R. J., and White, R. L. Suppression of malignant phenotype in a human prostate cancer cell line by fragments of normal chromosomal region 17q. Cancer Res, *55*: 3389-3394, 1995.

70. Berube, N. G., Speevak, M. D., and Chevrette, M. Suppression of tumorigenicity of human prostate cancer cells by introduction of human chromosome del(12)(q13). Cancer Res, *54*: 3077-3081, 1994.

71. Pienta, K. J., and Coffey, D. S. Nuclear-cytoskeletal interactions: evidence for

physical connections between the nucleus and cell periphery and their alteration by transformation. J Cell Biochem, *49*: 357-365, 1992.

72. Miele, M. E., Robertson, G., Lee, J. H., Coleman, A., McGary, C. T., Fisher, P. B., Lugo, T. G., and Welch, D. R. Metastasis suppressed, but tumorigenicity and local invasiveness unaffected, in the human melanoma cell line MelJuSo after introduction of human chromosomes 1 or 6. Mol Carcinog, *15*: 284-299, 1996.

73. You, J., Miele, M. E., Dong, C., and Welch, D. R. Suppression of human melanoma metastasis by introduction of chromosome 6 may be partially due to inhibition of motility, but not to inhibition of invasion. Biochem Biophys Res Commun, *208*: 476-484, 1995.

74. Miele, M. E., De La Rosa, A., Lee, J. H., Hicks, D. J., Dennis, J. U., Steeg, P. S., and Welch, D. R. Suppression of human melanoma metastasis following introduction of chromosome 6 is independent of NME1 (Nm23). Clin Exp Metastasis, *15*: 259-265, 1997.

75. Phillips, K. K., Welch, D. R., Miele, M. E., Lee, J. H., Wei, L. L., and Weissman, B. E. Suppression of MDA-MB-435 breast carcinoma cell metastasis following the introduction of human chromosome 11. Cancer Res, *56*: 1222-1227, 1996.

76. Nihei, N., Ohta, S., Kuramochi, H., Kugoh, H., Oshimura, M., Barrett, J. C., Isaacs, J. T., Igarashi, T., Ito, H., Masai, M., Ichikawa, Y., and Ichikawa, T. Metastasis suppressor gene(s) for rat prostate cancer on the long arm of human chromosome 7. Genes Chromosomes Cancer, *24*: 1-8, 1999.

77. Nihei, N., Ichikawa, T., Kawana, Y., Kuramochi, H., Kugoh, H., Oshimura, M., Hayata, I., Shimazaki, J., and Ito, H. Mapping of metastasis suppressor gene(s) for rat prostate cancer on the short arm of human chromosome 8 by irradiated microcell-mediated chromosome transfer. Genes Chromosomes Cancer, *17*: 260-268, 1996.

78. Kuramochi, H., Ichikawa, T., Nihei, N., Kawana, Y., Suzuki, H., Schalken, J. A., Takeichi, M., Nagafuchi, A., Ito, H., and Shimazaki, J. Suppression of invasive ability of highly metastatic rat prostate cancer by introduction of human chromosome 8. Prostate, *31*: 14-20, 1997.

79. Nihei, N., Ichikawa, T., Kawana, Y., Kuramochi, H., Kugo, H., Oshimura, M.,

Killary, A. M., Rinker-Schaeffer, C. W., Barrett, J. C., Isaacs, J. T., and et al. Localization of metastasis suppressor gene(s) for rat prostatic cancer to the long arm of human chromosome 10. Genes Chromosomes Cancer, *14*: 112-119, 1995.

80. Rinker-Schaeffer, C. W., Hawkins, A. L., Ru, N., Dong, J., Stoica, G., Griffin, C. A., Ichikawa, T., Barrett, J. C., and Isaacs, J. T. Differential suppression of mammary and prostate cancer metastasis by human chromosomes 17 and 11. Cancer Res, *54*: 6249-6256, 1994.

81. Ichikawa, T., Ichikawa, Y., Dong, J., Hawkins, A. L., Griffin, C. A., Isaacs, W. B., Oshimura, M., Barrett, J. C., and Isaacs, J. T. Localization of metastasis suppressor gene(s) for prostatic cancer to the short arm of human chromosome 11. Cancer Res, *52*: 3486-3490, 1992.

82. Mashimo, T., Watabe, M., Cuthbert, A. P., Newbold, R. F., Rinker-Schaeffer, C. W., Helfer, E., and Watabe, K. Human chromosome 16 suppresses metastasis but not tumorigenesis in rat prostatic tumor cells. Cancer Res, *58*: 4572-4576, 1998.

83. Backer, J. M., Mendola, C. E., Kovesdi, I., Fairhurst, J. L., O'Hara, B., Eddy, R. L., Jr., Shows, T. B., Mathew, S., Murty, V. V., and Chaganti, R. S. Chromosomal localization and nucleoside diphosphate kinase activity of human metastasis-suppressor genes NM23-1 and NM23-2. Oncogene, *8*: 497-502, 1993.

84. Kantor, J. D., McCormick, B., Steeg, P. S., and Zetter, B. R. Inhibition of cell motility after nm23 transfection of human and murine tumor cells. Cancer Res, *53*: 1971-1973, 1993.

85. Leone, A., Flatow, U., King, C. R., Sandeen, M. A., Margulies, I. M., Liotta, L. A., and Steeg, P. S. Reduced tumor incidence, metastatic potential, and cytokine responsiveness of nm23-transfected melanoma cells. Cell, *65*: 25-35, 1991.

86. Parhar, R. S., Shi, Y., Zou, M., Farid, N. R., Ernst, P., and al-Sedairy, S. T. Effects of cytokine-mediated modulation of nm23 expression on the invasion and metastatic behavior of B16F10 melanoma cells. Int J Cancer, *60*: 204-210, 1995.

87. de la Rosa, A., Williams, R. L., and Steeg, P. S. Nm23/nucleoside diphosphate kinase: toward a structural and biochemical understanding of its biological functions. Bioessays, *17*: 53-62, 1995.

88. Baba, H., Urano, T., Okada, K., Furukawa, K., Nakayama, E., Tanaka, H.,

Iwasaki, K., and Shiku, H. Two isotypes of murine nm23/nucleoside diphosphate kinase, nm23-M1 and nm23-M2, are involved in metastatic suppression of a murine melanoma line. Cancer Res, *55*: 1977-1981, 1995.

89. Lee, J. H., and Welch, D. R. Identification of highly expressed genes in metastasis-suppressed chromosome 6/human malignant melanoma hybrid cells using subtractive hybridization and differential display. Int J Cancer, *71*: 1035-1044, 1997.

90. Freije, J.M.P., Mac Donald, N.J., Steeg, P.S. Differential gene expression in tumor metastasis: Nm23 in Attempts to understand metastasis formation II: regulatory factors. Gunthert, U. Shlag, P. M. Birchmeier, W. Editors, Springer-Verlag, pp 215-232, 1996.

91. Freije, J. M., MacDonald, N. J., and Steeg, P. S. Nm23 and tumour metastasis: basic and translational advances. Biochem Soc Symp, *63*: 261-271, 1998.

92. Leone, A., Flatow, U., VanHoutte, K., and Steeg, P. S. Transfection of human nm23-H1 into the human MDA-MB-435 breast carcinoma cell line: effects on tumor metastatic potential, colonization and enzymatic activity. Oncogene, *8*: 2325-2333, 1993.

93. Bhujwalla, Z. M., Aboagye, E. O., Gillies, R. J., Chacko, V. P., Mendola, C. E., and Backer, J. M. Nm23-transfected MDA-MB-435 human breast carcinoma cells form tumors with altered phospholipid metabolism and pH: a 31P nuclear magnetic resonance study in vivo and in vitro. Magn Reson Med, *41*: 897-903, 1999.

94. Russell, R. L., Geisinger, K. R., Mehta, R. R., White, W. L., Shelton, B., and Kute, T. E. nm23--relationship to the metastatic potential of breast carcinoma cell lines, primary human xenografts, and lymph node negative breast carcinoma patients. Cancer, *79*: 1158-1165, 1997.

95. Fukuda, M., Ishii, A., Yasutomo, Y., Shimada, N., Ishikawa, N., Hanai, N., Nagata, N., Irimura, T., Nicolson, G. L., and Kimura, N. Decreased expression of nucleoside diphosphate kinase alpha isoform, an nm23-H2 gene homolog, is associated with metastatic potential of rat mammary-adenocarcinoma cells. Int J Cancer, *65*: 531-537, 1996.

96. Lim, S., Lee, H. Y., and Lee, H. Inhibition of colonization and cell-matrix adhesion after nm23-H1 transfection of human prostate carcinoma cells. Cancer Lett, *133*: 143-149, 1998.

97. Hsu, S., Huang, F., Wang, L., Banerjee, S., Winawer, S., and Friedman, E. The role of nm23 in transforming growth factor beta 1-mediated adherence and growth arrest. Cell Growth Differ, *5*: 909-917, 1994.

98. Miyazaki, H., Fukuda, M., Ishijima, Y., Takagi, Y., Iimura, T., Negishi, A., Hirayama, R., Ishikawa, N., Amagasa, T., and Kimura, N. Overexpression of nm23-H2/NDP kinase B in a human oral squamous cell carcinoma cell line results in reduced metastasis, differentiated phenotype in the metastatic site, and growth factor-independent proliferative activity in culture. Clin Cancer Res, *5*: 4301-4307, 1999.

99. Otsuki, K., Alcalde, R. E., Matsumura, T., and Kimura, N. Immunohistochemical analysis of nucleoside diphosphate kinases in oral squamous cell carcinomas. Oncology, *54*: 63-68, 1997.

100. Kawana, Y., Komiya, A., Ueda, T., Nihei, N., Kuramochi, H., Suzuki, H., Yatani, R., Imai, T., Dong, J. T., Yoshie, O., Barrett, J. C., Isaacs, J. T., Shimazaki, J., Ito, H., and Ichikawa, T. Location of KAI1 on the short arm of human chromosome 11 and frequency of allelic loss in advanced human prostate cancer. Prostate, *32*: 205-213, 1997.

101. Dong, J. T., Lamb, P. W., Rinker-Schaeffer, C. W., Vukanovic, J., Ichikawa, T., Isaacs, J. T., and Barrett, J. C. KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2 [see comments]. Science, *268*: 884-886, 1995.

102. Gao, A. C., Lou, W., Dong, J. T., and Isaacs, J. T. CD44 is a metastasis suppressor gene for prostatic cancer located on human chromosome 11p13. Cancer Res, *57*: 846-849, 1997.

103. Ueda, T., Ichikawa, T., Tamaru, J., Mikata, A., Akakura, K., Akimoto, S., Imai, T., Yoshie, O., Shiraishi, T., Yatani, R., Ito, H., and Shimazaki, J. Expression of the KAI1 protein in benign prostatic hyperplasia and prostate cancer. Am J Pathol, *149*: 1435-1440, 1996.

104. Rubinstein, E., Le Naour, F., Lagaudriere-Gesbert, C., Billard, M., Conjeaud, H., and Boucheix, C. CD9, CD63, CD81, and CD82 are components of a surface tetraspan network connected to HLA-DR and VLA integrins. Eur J Immunol, *26*: 2657-2665, 1996.

105. Berditchevski, F., and Odintsova, E. Characterization of integrin-tetraspanin adhesion complexes: role of tetraspanins in integrin signaling. J Cell Biol, *146*: 477-492, 1999.

106. Maecker, H. T., Todd, S. C., and Levy, S. The tetraspanin superfamily: molecular facilitators. Faseb J, *11*: 428-442, 1997.

107. Phillips, K. K., White, A. E., Hicks, D. J., Welch, D. R., Barrett, J. C., Wei, L. L., and Weissman, B. E. Correlation between reduction of metastasis in the MDA-MB-435 model system and increased expression of the Kai-1 protein. Mol Carcinog, *21*: 111-120, 1998.

108. Wei, L. L., Yang, X., Phillips, K., Weissman, B. E., and Welch, D. R. Proc. Am. Assoc. Cancer Res., *37*: 76, 1996.

109. Takaoka, A., Hinoda, Y., Sato, S., Itoh, F., Adachi, M., Hareyama, M., and Imai, K. Reduced invasive and metastatic potentials of KAI1-transfected melanoma cells. Jpn J Cancer Res, *89*: 397-404, 1998.

110. Takaoka, A., Hinoda, Y., Satoh, S., Adachi, Y., Itoh, F., Adachi, M., and Imai, K. Suppression of invasive properties of colon cancer cells by a metastasis suppressor KAI1 gene. Oncogene, *16*: 1443-1453, 1998.

111. Lombardi, D. P., Geradts, J., Foley, J. F., Chiao, C., Lamb, P. W., and Barrett, J. C. Loss of KAI1 expression in the progression of colorectal cancer [In Process Citation]. Cancer Res, *59*: 5724-5731, 1999.

112. West, A., Vojta, P. J., Welch, D. R., and Weissman, B. E. Chromosome localization and genomic structure of the KiSS-1 metastasis suppressor gene (KISS1). Genomics, *54*: 145-148, 1998.

113. Lee, J. H., Miele, M. E., Hicks, D. J., Phillips, K. K., Trent, J. M., Weissman, B. E., and Welch, D. R. KiSS-1, a novel human malignant melanoma metastasis-suppressor gene [see comments] [published erratum appears in J Natl Cancer Inst 1997 Oct 15;89(20):1549]. J Natl Cancer Inst, *88*: 1731-1737, 1996.

114. Lee, J. H., and Welch, D. R. Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene, KiSS-1. Cancer Res, *57*: 2384-2387, 1997.

115. Ferha, M. J., Samant, R. S., Verderame, M. F., and Welch, D. R. Functional evidence for a novel human breast carcinoma metastasis-suppressor, BrMS1, encoded at chromosome 11q13 [In Process Citation]. Cancer Research, *60*: 2000.

116. White, R. A., Hughes, R. T., Adkison, L. R., Bruns, G., and Zon, L. I. The gene encoding protein kinase SEK1 maps to mouse chromosome 11 and human chromosome 17. Genomics, *34*: 430-432, 1996.

117. Yoshida, B. A., Dubauskas, Z., Chekmareva, M. A., Christiano, T. R., Stadler, W. M., and Rinker-Schaeffer, C. W. Mitogen-activated protein kinase kinase 4/stress-activated protein/Erk kinase 1 (MKK4/SEK1), a prostate cancer metastasis suppressor gene encoded by human chromosome 17 [In Process Citation]. Cancer Res, *59*: 5483-5487, 1999.

118. Whitmarsh, A. J., and Davis, R. J. Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. J Mol Med, *74*: 589-607, 1996.

119. Ip, Y. T., and Davis, R. J. Signal transduction by the c-Jun N-terminal kinase (JNK)--from inflammation to development. Curr Opin Cell Biol, *10*: 205-219, 1998.

120. Gunthert, U., Stauder, R., Mayer, B., Terpe, H. J., Finke, L., and Friedrichs, K. Are CD44 variant isoforms involved in human tumour progression? Cancer Surv, *24*: 19-42, 1995.

121. Lou, W., Krill, D., Dhir, R., Becich, M. J., Dong, J. T., Frierson, H. F., Jr., Isaacs, W. B., Isaacs, J. T., and Gao, A. C. Methylation of the CD44 metastasis suppressor gene in human prostate cancer. Cancer Res, *59*: 2329-2331, 1999.

122. Noordzij, M. A., van Steenbrugge, G. J., Schroder, F. H., and Van der Kwast, T. H. Decreased expression of CD44 in metastatic prostate cancer. Int J Cancer, *84*: 478-483, 1999.

123. Nagabhushan, M., Pretlow, T. G., Guo, Y. J., Amini, S. B., Pretlow, T. P., and Sy, M. S. Altered expression of CD44 in human prostate cancer during progression.

Am J Clin Pathol, *106*: 647-651, 1996.

124. De Marzo, A. M., Bradshaw, C., Sauvageot, J., Epstein, J. I., and Miller, G. J. CD44 and CD44v6 downregulation in clinical prostatic carcinoma: relation to Gleason grade and cytoarchitecture. Prostate, *34*: 162-168, 1998.

125. Noordzij, M. A., van Steenbrugge, G. J., Verkaik, N. S., Schroder, F. H., and van der Kwast, T. H. The prognostic value of CD44 isoforms in prostate cancer patients treated by radical prostatectomy. Clin Cancer Res, *3*: 805-815, 1997.

126. Kallakury, B. V., Sheehan, C. E., Ambros, R. A., Fisher, H. A., Kaufman, R. P., Jr., Muraca, P. J., and Ross, J. S. Correlation of p34cdc2 cyclin-dependent kinase overexpression, CD44s downregulation, and HER-2/neu oncogene amplification with recurrence in prostatic adenocarcinomas. J Clin Oncol, *16*: 1302-1309, 1998.

127. Sneath, R. J., and Mangham, D. C. The normal structure and function of CD44 and its role in neoplasia. Mol Pathol, *51*: 191-200, 1998.

128. Schneider, S. S., Schick, C., Fish, K. E., Miller, E., Pena, J. C., Treter, S. D., Hui, S. M., and Silverman, G. A. A serine proteinase inhibitor locus at 18q21.3 contains a tandem duplication of the human squamous cell carcinoma antigen gene. Proc Natl Acad Sci U S A, *92*: 3147-3151, 1995.

129. Zou, Z., Anisowicz, A., Hendrix, M. J., Thor, A., Neveu, M., Sheng, S., Rafidi, K., Seftor, E., and Sager, R. Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells [see comments]. Science, *263*: 526-529, 1994.

130. Seftor, R. E., Seftor, E. A., Sheng, S., Pemberton, P. A., Sager, R., and Hendrix, M. J. maspin suppresses the invasive phenotype of human breast carcinoma. Cancer Res, *58*: 5681-5685, 1998.

131. Umekita, Y., Hiipakka, R. A., and Liao, S. Rat and human maspins: structures, metastatic suppressor activity and mutation in prostate cancer cells. Cancer Lett, *113*: 87-93, 1997.

132. Pienta, K. J., Partin, A. W., and Coffey, D. S. Cancer as a disease of DNA organization and dynamic cell structure. Cancer Res, *49*: 2525-2532, 1989.

133. Guo, X., Friess, H., Graber, H. U., Kashiwagi, M., Zimmermann, A., Korc, M., and Buchler, M. W. KAI1 expression is up-regulated in early pancreatic cancer and decreased in the presence of metastases. Cancer Res, *56*: 4876-4880, 1996.

134. Guo, X. Z., Friess, H., Di Mola, F. F., Heinicke, J. M., Abou-Shady, M., Graber, H. U., Baer, H. U., Zimmermann, A., Korc, M., and Buchler, M. W. KAI1, a new metastasis suppressor gene, is reduced in metastatic hepatocellular carcinoma. Hepatology, *28*: 1481-1488, 1998.

135. Yu, Y., Yang, J. L., Markovic, B., Jackson, P., Yardley, G., Barrett, J., and Russell, P. J. Loss of KAI1 messenger RNA expression in both high-grade and invasive human bladder cancers. Clin Cancer Res, *3*: 1045-1049, 1997.

136. Adachi, M., Taki, T., Ieki, Y., Huang, C. L., Higashiyama, M., and Miyake, M. Correlation of KAI1/CD82 gene expression with good prognosis in patients with non-small cell lung cancer. Cancer Res, *56*: 1751-1755, 1996.

137. Uchida, S., Shimada, Y., Watanabe, G., Li, Z. G., Hong, T., Miyake, M., and Imamura, M. Motility-related protein (MRP-1/CD9) and KAI1/CD82 expression inversely correlate with lymph node metastasis in oesophageal squamous cell carcinoma. Br J Cancer, *79*: 1168-1173, 1999.

138. Geradts, J., Maynard, R., Birrer, M. J., Hendricks, D., Abbondanzo, S. L., Fong, K. M., Barrett, J. C., and Lombardi, D. P. Frequent loss of KAI1 expression in squamous and lymphoid neoplasms. An immunohistochemical study of archival tissues. Am J Pathol, *154*: 1665-1671, 1999.

139. Chekmareva, M. A., Kadkhodaian, M. M., Hollowell, C. M., Kim, H., Yoshida, B. A., Luu, H. H., Stadler, W. M., and Rinker-Schaeffer, C. W. Chromosome 17-mediated dormancy of AT6.1 prostate cancer micrometastases. Cancer Res, *58*: 4963-4969, 1998.

140. Holmgren, L., O'Reilly, M. S., and Folkman, J. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression [see comments]. Nat Med, *1*: 149-153, 1995.

141. Welch, D. R., Chen, P., Miele, M. E., McGary, C. T., Bower, J. M., Stanbridge, E. J., and Weissman, B. E. Microcell-mediated transfer of chromosome 6 into metastatic human C8161 melanoma cells suppresses metastasis but does not inhibit tumorigenicity. Oncogene, *9*: 255-262, 1994.

142. Miele, M. E., Jewett, M. D., Goldberg, S. F., Hyatt, D. L., Morelli, C., Gualandi, F., Rimessi, P., Hicks, D. J., Weissman, B. E., Barbanti-Brodano, G., and Welch,

D. R. A human melanoma metastasis-suppressor locus maps to 6q16.3-q23. International Journal of Cancer, in press.

143. Welch, D. R., and Goldberg, S. F. Molecular mechanisms controlling human melanoma progression and metastasis. Pathobiology, *65*: 311-330, 1997.

144. Goldberg, S. F., Harms, J. F., Quon, K., and Welch, D. R. Metastasis-suppressed C8161 melanoma cells arrest in lung but fail to proliferate. Clinical and Experimental Metastasis, *in press*: 2000.

145. Nelson, P. S., Ng, W. L., Schummer, M., True, L. D., Liu, A. Y., Bumgarner, R. E., Ferguson, C., Dimak, A., and Hood, L. An expressed-sequence-tag database of the human prostate: sequence analysis of 1168 cDNA clones. Genomics, *47*: 12-25, 1998.

146. Hawkins, V., Doll, D., Bumgarner, R., Smith, T., Abajian, C., Hood, L., and Nelson, P. S. PEDB: the Prostate Expression Database. Nucleic Acids Res, *27*: 204-208, 1999.

147. Bissell, M. J., Weaver, V. M., Lelievre, S. A., Wang, F., Petersen, O. W., and Schmeichel, K. L. Tissue structure, nuclear organization, and gene expression in normal and malignant breast. Cancer Res, *59*: 1757-1763s; discussion 1763s-1764s, 1999.

148. Boudreau, N., and Bissell, M. J. Extracellular matrix signaling: integration of form and function in normal and malignant cells. Curr Opin Cell Biol, *10*: 640-646, 1998.

149. Hayward, S. W., Grossfeld, G. D., Tlsty, T. D., and Cunha, G. R. Genetic and epigenetic influences in prostatic carcinogenesis (review). Int J Oncol, *13*: 35-47, 1998.

150. Baskin, L. S., Hayward, S. W., DiSandro, M. S., Li, Y. W., and Cunha, G. R. Epithelial-mesenchymal interactions in the bladder. Implications for bladder augmentation [In Process Citation]. Adv Exp Med Biol, *462*: 49-61, 1999.

151. Olumi, A. F., Grossfeld, G. D., Hayward, S. W., Carroll, P. R., Tlsty, T. D., and Cunha, G. R. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. Cancer Res, *59*: 5002-5011, 1999.

152. Chung, L. W., and Davies, R. Prostate epithelial differentiation is dictated by its

surrounding stroma. Mol Biol Rep, *23*: 13-19, 1996.

153. Chung, L. W. The role of stromal-epithelial interaction in normal and malignant growth. Cancer Surv, *23*: 33-42, 1995.

154. Chung, L. W. Implications of stromal-epithelial interaction in human prostate cancer growth, progression and differentiation. Semin Cancer Biol, *4*: 183-192, 1993.

155. Welch, D. R. Technical considerations for studying cancer metastasis in vivo. Clin Exp Metastasis, *15*: 272-306, 1997.

156. Day, M. L., Foster, R. G., Day, K. C., Zhao, X., Humphrey, P., Swanson, P., Postigo, A. A., Zhang, S. H., and Dean, D. C. Cell anchorage regulates apoptosis through the retinoblastoma tumor suppressor/E2F pathway. J Biol Chem, *272*: 8125-8128, 1997.

157. Kendrew, J., The Thread of life: an introduction to molecular biology, 1996, Cambridge, MA: Harvard University Press.

158. Schrodinger, E., What is life?: the physical aspect of the living cell; with Mind and matter: & autobiographical sketches. Canto ed. Cambridge paperbacks. 1992 New York: Cambridge University Press.

159. Coffey, D. S. Self-organization, complexity and chaos: the new biology for medicine [see comments]. Nat Med, *4*: 882-885, 1998.

160. Schwab, E.D. and K.J. Pienta, Explaining aberrations of cell structure and signaling in cancer using complex adaptive systems. Advances in Molecular and Cell Biology *24*: 207-247, 1997

Figure Legends

Figure 1. Development of spontaneous hematogenous metastases. **Panel A.** The development of spontaneous hematogenous metastases requires cancer cells to complete a well-defined series of steps. This figure is adapted from reference 11. **Panel B** In order to form overt metastases, disseminated cells must complete additional steps at the metastatic site(s).

Figure 2. Identification of metastasis-suppressor activities using microcell mediated chromosomal transfer. A9 donor cells containing a single human chromosome are used for the preparation of microcells that will be transferred to the recipient cell lines. Stable microcell hybrids are selected and characterized by molecular and cellular methods. Inclusion of a variety of controls is critical for the definition of metastasis-suppressor activity *in vivo*. The potential outcomes of *in vivo* studies using control, suppressed and unsuppressed hybrids are illustrated at left.

Figure 3. Examination of the mechanism of metastasis suppression by chromosome 17 and 6. **Panel A.** Quantification of overt surface metastases and micrometastases. AT6.1 cells are highly metastatic rat prostate cancer cells. AT6.1-17-Tβgal cells contain the metastasis-suppressor region of human chromosome 17 and are tagged with a β-galactosidase reporter gene enabling the sensitive detection of microscopic metastases. The numbers of overt and microscopic metastases were determined using Bouin's and X-gal staining respectively. At the experimental endpoint, lungs were removed from tumor bearing animals. *Left*, lung from AT6.1 tumor-bearing animal stained with Bouin's solution; *Middle*, lung from AT6.1-17-Tβgal tumor-bearing animal stained with Bouin's solution; and *Right*, lung from AT6.1-17-Tβgal tumor-bearing animal stained for βgalactosidase activity. The average number of overt or microscopic metastases and SE are shown below the panels. This Figure is adapted from reference 115. **Panel B.** A combination of techniques has been used to examine the timecourse of cancer cell

dissemination and growth in suppressed AT6.1-17 cells as compared to metastatic AT6.1 parental cells. These data indicate that genes encoded by chromosome 17 inhibit a step in metastatic colonization. ***Panel C.*** Photomicrographs of mouse lung following intravenous injection of GFP-tagged C8161 and metastasis-suppressed neo6/C8161 cells (Panel C3) are present. At one month, however, C8161 cells have proliferated to form macroscopic lung lesions (Panel C2); but most neo6/C8161 cells have been cleared. Occasional single cells (Panel C4, arrows) can be found in the lungs, but fail to proliferate. These results imply that chromosome 6 suppresses metastasis by inhibiting the ability of C8161 cells to grow in the lung at an early stage of colonization. Data adapted from [120].

Table 1. Chromosomal regions identified by MMCT that suppress metastases *in vivo*

Chromosomal Location	Tumor Types	Cell Lines Tested[1]	In Vitro Phenotype[2]	In Vivo Phenotype
chromosome 1	melanoma [72]	MelJuSo (hu)	ND	↓ spont. mets. ↓ exp. mets.
6q16.3-q23 chromosome 6	melanoma [73, 74] breast [75]	C8161 (hu) MelJuSo (hu) MDA-MB-435 (hu)	↓ motility ND ND	↓ spont. mets. ↓ exp. mets. occasional single cells (detected by GFP tagging) which are growth suppressed but viable ↓ spont. mets. ↓ exp. mets. NE spont. mets.
7q21-22 and/or 7q31.2-32	prostate [76]	AT6.3 (rt)	ND	↓ spont. mets. ↓ exp. mets.
8p21-p12	prostate [77, 78]	AT6.2 (rt)	↓ invasion	↓ spont. mets. NE exp. mets.
10cen-10q23	prostate [79]	AT6.3 (rt)	ND	↓ spont. mets.
11q13.1-13.2 11pter-q14	breast [75, 80]	MDA-MB-435 (hu) R1564 (rt)	ND ND	↓ spont. mets. NE spont. mets.
11p11.2-13	prostate [68]	AT6.1 (rt) AT3.1 (rt)	ND ND	↓ spont. mets. ↓ spont. mets.
12qcen-q13 and/or 12q24-ter	prostate [64]	AT6.1	NE motility[3] NE invasion[3]	↓ spont. mets. no micrometastases observed at the experimental endpoint
16q24.2	prostate [82]	AT6.1	ND	↓ spont. mets.
17p12-11.2and/or 17cen-q12	prostate [63]	AT6.1	NE motility[3] NE invasion[3]	↓ spont. mets. micrometastases observed at the experimental endpoint

[1] The species of origin is indicated for each cell line; (hu), human, (rt), rat; references shown in brackets.

[2] Motility was measured by micropipet motility assay or by migration towards a chemoattractant in Boyden   chambers. Invasion was measured by migration through Matrigel.

[3] Rinker-Schaeffer, C. W., unpublished results.

Table 2. Summary of metastasis-suppressor genes identified

Metastasis Suppressor Gene	Discovery Method	Tumor Types	Cell Lines Transfected[2]	In Vitro Phenotype[2]	In Vivo Phenotype	Status in Clinical Disease	Reported Mechanisms of Action
nm23[3] (NME1) (17q21.3) [83]	cDNA subtraction	**melanoma** [84-89]	K-1735 (mu)	↓ motility ↓colony formation ↓prolif. (TGFβ)	↓exp. mets.	inverse correlation between Nm23 expression and metastatic potential	• nucleotide diphosphate kinase • signal transduction • transcriptional activation [90-91]
			B16 F10 (mu)	↓ invasiveness ↑ cell-cell adhesion ↑ immuno-sensitivity	↓exp. mets.		
			B16 FE7 (mu)	ND	↓exp. mets.		
			MelJuSo (hu)	ND	↓exp. mets.		
		breast [84, 87, 92-95]	MDA-MB-435 (hu)	↓ motility ↓colony formation	↓spont. mets.	inverse correlation between Nm23 expression and metastatic potential	
			MTLn3 (rt)	ND	↓spont. mets.		
		prostate [87, 96]	DU145 (hu)	↓colony formation ↓ invasiveness ↓ adhesion to extracellular matrix components	ND	no trend observed	
		colon [87, 97]	HD3[4] (hu) (AS-oligo study)	↓ adhesion to tissue culture dish ↓ growth arrest ↓ differentiation	ND	aggressive colorectal cancers have high expression of mutated Nm23	
			U9[4] (hu) (AS-oligo study)	No change	ND		
		oral [98, 99]	LMF4	ND	↓exp. mets. ↑differentiation	inverse correlation between Nm23	

KAI1[5] (11p11.2) also known as CD82 [100]	MMCT/Alu-specific PCR/ hybridization of cDNA library					expression and metastatic potential	• integrin signaling • cell adhesion • motility [104-106]
		prostate [33, 100-103]	AT6.1 (rt)	→ invasiveness	→ spont.mets.	inverse correlation bewteen protein expression and metastatic potential	
			AT3.1 (rt)	ND	NE spont.met.		
			AT6.3 (rt)	ND	→ spont. mets.		
		breast [32, 75, 107, 108]	MDA-MB-435 (hu)				
			• ch 11 MCT	→ invasiveness	→ spont. mets.	inverse correlation between protein expression and metastatic potential	
			• KAI1 cDNA transfection	ND	protein expression / modification in the 1° tumors and mets		
		melanoma [109]	MelJuSo (hu)	ND	→ exp. mets.[6]	ND	
			B16-BL6 (mu)	↑ cell aggregation → motility → invasiveness	→ exp. mets.		
		colon [110-111]	BM314 (hu)	↑ cell aggregation → motility → invasiveness	ND	inverse correlation between protein expression and metastatic potential	
			DLD-1 (hu)	↑ cell aggregation → motility → invasiveness	ND		

Gene	Method	Cancer type	Cell line	in vitro effects	metastasis	clinical	function
KiSS1 (1q32) [112]	MMCT/ cDNA subtraction	melanoma [89, 113, 114]	C8161	NE adhesion to extracellular matrix components NE invasion	↓exp. mets. ↓spont. mets.	ND	• signal transduction [113]
			MelJuSo	ND	↓exp. mets. ↓spont. mets.		
		breast [114]	MDA-MB-435	↓colony formation ↑ spread on collagen type IV NE motility	↓spont. mets.	ND	
BrMS1 (11q13.1-2) [115]	MMCT/ differential display	breast [115]	MDA-MB-435 (hu)	ND	→ spont. mets.	ND	• cell communicatio n • motility [115]
			MDA-MB-231 (hu)	ND	→ exp. mets.		
MKK4 (MAP2K4) (17p11.2) [116]	MMCT/ positional EST identification	prostate [117]	AT6.1 (rt)	ND	→ spont. mets.	ND	• cytokine/stres s-induced signal transduction [118-119]
CD44[i] (11p13) [120]	MMCT	prostate [102, 121-126]	AT3.1 (rt)	ND	→ spont. mets.	down-regulation of CD44 correlates with higher tumor grade, aneuploidy, and presence of distant metastases [127]	• receptor for both hyaluronic acid and osteopontin • cell adhesion [127]
maspin[i] (PI5) (18q21.3) [128]	subt. hybrid./ differential display	breast [129-130]	MDA-MB-435 (hu)	→ invasiveness → motility	→ 1° tumor growth	ND [no cohort studies, although weak expression in malignant cells of invasive breast carcinomas has	• serine protease inhibitor • modulation of integrin expression [130]

prostate [131]	AT3.1 (rt)	ND	NE 1° tumor growth NE spont. mets.	ND	been reported] ND

[1] The species of origin is indicated for each cell line; (mu), murine, (hu), human, (rt), rat.

[2] Cell motility was determined in chemotaxis assays using Boyden chambers, in phagokinetic track assays on cover slips, or by cinematography studies. Invasion was measured by migration through Matrigel or reconstituted basement membranes in Boyden chambers. Colony formation was evaluated in soft agar. Cell proliferation was measured by counting viable cells using a hemocytometer. Cell adhesion was evaluated by the ability of cells to form conjugates with lymphokine activated killer cells (LAK), the ability to adhere to tissue culture plates coated with laminin, fibronectin, collagen type I, or collagen type IV in the absence of fetal bovine serum (FBS), or by the ability to remain adherent to tissue culture plates after the removal of FBS and the addition of oligonucleotides and transforming growth factor beta (TGFβ). Immunosensitivity was determined in chromium release assays with LAK cells. Cell aggregation was examined by culturing single cell suspensions in Puck's saline + 0.8% fetal bovine serum. Cell spreading over extracellular matrix substrates was monitored over time by photography.

[3] Additional clinical studies have examined the expression of Nm23 in hepatocellular, gastric, ovarian, and cervical carcinomas (87).

[4] HD3 and U9 are sublines of the human colon carcinoma line, HT29, and differ in their responses to TGFβ.

[5] An inverse correlation between KAI1 protein and/or mRNA expression and malignant potential have been observed in pancreatic, nonsmall cell lung, bladder, hepatocellular, and oesophageal squamous cell carcinomas.

[6] Unpublished observations. (Welch, D.R.)

* does not fit the classic definition of a metastasis-suppressor gene

Metastasis suppressor gene

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Definition

A gene which blocks **metastasis** without affecting **tumorigenicity**. The suppressing activity could be the result of alterations at any of the steps in the **metastatic cascade**.

Molecular Characteristics

Although the definition of a metastasis suppressor gene would, at first, appear straightforward, it is important to emphasize the distinctions between tumorigenesis, tumor progression, invasion and metastasis. This section will briefly highlight the key aspects of those differences, but readers are encouraged to refer to individual entries for a more complete understanding of the terms.

Tumorigenesis refers to a cell's ability to proliferate continuously in the absence of persistent stimulation by the triggering carcinogenic agent(s). **Tumor progression** is the evolution of already tumorigenic cells (populations) towards increasing malignancy. **Invasion** is the migration of tumor cells away from the primary tumor mass. This process can involve the breakdown of physical barriers (e.g., basement membranes) by secretion of **proteinases** (i.e., protein-degrading enzymes). **Metastasis** is the process by which a tumor cell(s) spreads to other sites in the body and establishes a secondary tumor colony. The process is complex and involves many steps (i.e., the **metastatic cascade**).

The genetics of metastasis can be conceptualized as consisting of two components – positive and negative regulators. The positive regulators (i.e., metastasis promoting genes) drive metastasis formation. These are genes which, when expressed enhance the ability of a cell to complete one or more steps in the metastatic cascade. An example is the **MMPs** (matrix metalloproteinases) which are involved in enzymatic breakdown of basement membrane matrices. It is important to note that most metastasis promoting genes are neither necessary (because of redundancy) nor sufficient (because of the multiple steps in the metastatic cascade) to confer metastatic competency upon cells. Two genes – **RAS** and **MEK1**, however, do confer both tumorigenic and metastatic potential upon NIH3T3 cells.

In contrast, metastasis suppressor genes inhibit metastasis. Since metastasis requires cells to complete every step in the metastatic cascade, these genes can block any step(s) in the process. The first metastasis suppressor gene was discovered in 1984. Since that time, several candidate genes have been identified, but only six have been shown to suppress metastasis *in vivo* – **NME1**, **KiSS1**, **KAI1**, **E-cadherin**, **BRMS1**, and **MKK4**. Please refer to individual entries for more details.

Metastasis suppressor genes and metastasis promoting genes are analogous to **tumor**

suppressor genes and **oncogenes**, but there are important distinctions. *Tumor* suppressor genes dominantly inhibit tumor formation when wild-type expression is restored in a neoplastic cell. By definition, then, metastasis would also be suppressed (since the cells are nontumorigenic). *Metastasis* suppressor genes, on the other hand, block only the ability to form metastases. Restoring expression of a metastasis-suppressor would yield cells which are still tumorigenic, but which are no longer metastatic.

How are metastasis suppressor genes identified?

Two general approaches have been used to identify metastasis-controlling genes. The first involves comparison of gene expression in poorly or nonmetastatic cells with matched metastasis-competent cells. The specific techniques employed are **differential display** and **subtractive hybridization**. The second takes advantage of clinical observations that identified nonrandom chromosomal changes that occur during tumor progression. This information localized the gene(s) from which cloning could commence. Based upon karyotypic patterns observed in human cancers, additional metastasis suppressor genes to those listed above are hypothesized to exist; however, the identities of the specific genes have yet to be determined.

Clinical Relevance

The existence of genes that block metastasis implies that the metastasis could be theoretically controlled by agents which regulate these genes or mimic their behavior. However, gene therapy is not yet possible. In the meantime, however, the differential expression of metastasis-associated genes is being used by pathologists to more accurately define the cancers.

References:

Welch, D.R. and Rinker-Schaeffer, C.W. (1999) What defines a useful marker of metastasis in human cancer? Journal of the National Cancer Institute 91: 1351-1353.

Welch, D.R., Steeg, P.S., and Rinker-Schaeffer, C.W., (2000) Genetic regulation of human breast carcinoma metastasis. Biology of Breast Cancer Metastasis (In press).

BRMS1

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Definition:

BRMS1 [**B**reast **M**etastasis **S**uppressor **1**] is a human **metastasis suppressor gene** which, when over expressed, suppresses metastasis of human breast carcinoma cell lines in immunocompromised mouse models.

Molecular Characteristics

BRMS1 is located on chromosome 11q13.1-q13.2. It is spread over 10 Kb and is comprised of 10 exons, the first exon being untranslated. *BRMS1* cDNA is 1485 base pairs and encodes a novel protein of 246 amino acids (Mr~ 28.5 kDa). It is fairly conserved across species with it mouse homolog [*brms1*] having 95% homology at amino acid level. BRMS1 protein sequence shows presence of two nuclear localization sequences, two coiled-coil motifs and imperfect leucine zippers. It also shows presence of acid rich N-terminus and a potential endoplasmic retention signal. Thus, it shows characteristics of a transcription factor, but this function has yet to be definitively established. BRMS1 expression is not correlated with expression of other known metastasis suppressor genes viz. Nm23, Kai 1, KiSS1 and E-cadherin suggesting its involvement in a novel metastasis suppressor cascade.

Cellular and functional characteristics

BRMS1 shows almost ubiquitous expression in human tissues, with highest expression in kidney, placenta, peripheral blood lymphocytes and testis. Lowest expression is in brain and lung. Subcellular fractionation and immunofluorescence studies have determined that BRMS1 protein is predominantly nuclear. BRMS1 is involved in the establishment of cell-cell communication via gap-junctions, which is evident from the re-establishment of **gap junctional intercellular communication** in MDA-MB-435 and MDA-MB-231 human breast carcinoma cell lines. Wound healing studies performed in the same cell lines revealed an inverse effect of BRMS1 expression on cell **motility.** These results suggest that BRMS1 exerts complex changes on the breast carcinoma cells. Whichever changes in cell behavior are responsible for metastasis suppression will require further experimentation.

Clinical Relevance:

Survival rates and quality of life in breast cancer patients is significantly worse for patients with stage IV (metastatic) disease. Thus, decreased morbidity or mortality depends upon prevention and /or effective treatment of metastatic disease. Karyotypic alterations such as deletions or translocations are frequent in cancerous cells. Among the most common changes (40-65% of cases) occurring in breast carcinoma are deletions and amplifications near band 11q13. This stresses the importance of the region encoding or adjacent to BRMS1. Now that BRMS1 has been demonstrated to suppress metastasis in animal models, further studies will be needed to

determine whether this is the only gene involved at that location.

References

Welch, D.R., Steeg, P.S., and Rinker-Schaeffer, C.W., (2000) Genetic regulation of human breast carcinoma metastasis. Biology of Breast Cancer Metastasis (In press).

Welch, D.R., Seraj, M.J., Samant, R.S., Leonard, T.O., Harms, J.F., Verderame, M.F.

A human breast cancer metastasis suppressor gene encoded on chromosome 11q13.1-q13.2.

Introduction of normal human chromosome 11 reduces the metastatic capacity of MDA-MB-435 human breast carcinoma cells without affecting tumorigenicity [Phillips *et al*. (1996) *Cancer Res*. 56: 1222-7]. This suggests the presence of a metastasis suppressor genes on human chromosome 11. Differential display was done to identify mRNAs with increased expression in neo11/435 hybrids compared with their metastatic counterparts. Six cDNA fragments were consistently differentially expressed in replicate amplifications and RNA analyses at levels at least 5-fold greater in metastasis-suppressed neo11/435 hybrids. Three of the six candidates were homologous to known cDNAs. The remaining three had minimal homology to known sequences or ESTs. We isolated a full-length cDNA for one of the novel genes, designated *BRMS1* (**Breast**-cancer **M**etastasis **S**uppressor **1**), which maps to human chromosome 11q13.1-q13.2 by fluorescence *in situ* hybridization. *BRMS1*-transfected human breast carcinoma cell lines MDA-MB-435 and MDA-MB-231 formed significantly fewer metastases in athymic mice than parental or vector-only controls in an expression-dependent manner. Like the neo11/435 hybrids, *BRMS1* transfectants remain tumorigenic. These results provide functional evidence that *BRMS1* is a metastasis-suppressor. BRMS1 predicted protein sequence contains regions with homology to DNA binding domains, coiled-coil, leucine zipper, nuclear localization and some consensus phosphorylation sites. These homologies suggest that BRMS1 may function in a signaling cascade as a transcription factor. The mechanism by which *BRMS1* suppresses metastasis is not fully elucidated, but does not involve upregulation of Nm23-H1, KiSS1 or KAI1 metastasis-suppressor genes.

BrMS1 - a human breast carcinoma metastasis-suppressor gene.

Samant, R.S., Seraj, M.J., Meehan, W.J., Harms, J.F., Leonard, T.O., Shevde, L.A., Sakamaki, T., Winter, C.R., Verderame, M.F., and Welch, D.R.

Microcell-mediated introduction of chromosome 11 suppresses MDA-MB-435 (435) metastasis without affecting tumorigenicity, suggesting presence of a metastasis suppressor gene. Differential display comparing 435 with neo11/435 identified six putative cDNAs with <gt>5 fold higher expression in the latter. One novel, differentially expressed gene, *BRMS1*, mapped to human chromosome 11q13.1-q13.2 by FISH. *BRMS1*- transfected 435 and MDA-MB-231 were suppressed for metastasis but not tumorigenicity. Epitope-tagged BRMS1 localizes to the nucleus by fluorescence microscopy and cellular fractionation. Presence of coiled coil and leucine zipper motifs suggest involvement in transcription, yet Nm23, E-Cad, Kail and KiSS1 metastasis-suppressor gene expression is not upregulated in transfectants. These results provide functional evidence for a breast cancer metastasis suppressor, *BRMS1*, encoded on chromosome 11 in a region frequently altered in late stage human breast carcinoma. *Support: DAMD17-96-6152, CA62168, Natl. Fndn. Cancer Res.*

GENOMIC ORGANIZATION AND CHROMOSOMAL LOCALIZATION OF THE BREAST METASTASIS SUPPRESSOR GENE [BRMS1] R.S. Samant; M.T. Debies, M.J. Seraj, M.F. Verderame, D.R. Welch. Jake Gittlen Cancer Research Inst., Penn State College of Medicine, Hershey, PA 17033.

We recently cloned a novel metastasis suppressor gene (*BRMS1*) by differential display comparing the metastatic breast carcinoma cell line MDA-MB-435 to metastasis suppressed human chromosome 11 hybrids with MDA-MB-435. A BAC clone [412(n24)] was confirmed to encode the entire *BRMS1* gene. Fluorescence *in situ* hybridization using 412(n24) as a probe revealed that BRMS1 maps to chromosome 11q13.1-q13.2. Detailed analysis of *BRMS1* genomic DNA using overlapping subclones and PCR shows that the gene spans over 10kb and is organized into 10 exons and 9 introns. Exon 1 is untranslated. The region 5' of exon1 was characterized for regulatory promoter elements and showed presence of potential binding sites for SP1, AP-2 and c-Myb. We observed that the 5' region also includes another gene iGnT, (i-β-1,3-N-acetylglucos-aminyltransferase)[Sasaki et al.,(1997) *PNAS*, 94:14294], which is oriented head to tail. Taken together, these results suggest the possibility that, iGnT and BRMS1 transcriptional controls may be linked.

Metastatic breast carcinoma correlates with a breakdown in gap junction function and expression
Saunders,M.M.; Li,Z.; Winter,C.R.; Welch,D.R.; Donahue,H.J.
Clinical and Experimental Metastasis (in press)

Gap junctional intercellular communication (GJIC) is believed to play a role in the growth and maintenance of normal cell function. While breakdown of GJIC has been previously linked to tumorigenesis, its role in metastasis has not been clearly defined. In this study we examined heterotypic GJIC function and expression using the highly metastatic MDA-MB-435 (435), a transfected clone containing the metastasis-suppressor gene, BrMS1 (435/BrMS1) and a vector control (435pVC). The 435 and 435/BrMS1 cell lines while exhibiting markedly different levels of metastatic potential are equally tumorigenic and we have previously shown that the 435 cell line is not homotypically coupled while the 435/BrMS1 line is highly coupled. To investigate heterotypic GJIC we used fluorescent dye assays to examine coupling between the various combinations of tumorigenic cell lines, as well as their ability to communicate with a breast tissue cell line (Hs578Bst). Connexin (Cx) 43, 45 and 46 mRNA and protein expression were quantified using RT-PCR and Western blot analysis. From functional dye experiments, we found that none of the tumorigenic cell lines were able to communicate with each other. The 435 and 435pVC also did not communicate with the Hs578Bst cell line while the 435/BrMS1 was highly coupled to these normal breast tissue cells. Hs578Bst highly expressed Cx 43 mRNA and protein, whereas none of the tumorigenic cell lines, regardless of metastatic potential expressed mRNA or protein for Cx 43, 45 or 46. Taken in total with our previous findings, this work supports the hypothesis that breakdown in gap junction function or expression is important not only for maintenance of a malignant phenotype in tumorigenesis, but that the restoration of homotypic and heterotypic GJIC via the introduction of a breast metastasis-suppressor gene into a highly metastatic cell line significantly reduces metastasis.